Microbial Bile Acid Metabolism Shapes T Cell Responses During Inflammation

Oriana Miltiadous

2023-08-07

Contents

Chapter 1

Introduction: load the datasets

1.1 Load packages

```
library(janitor)
library(readxl)
library(tidyverse)
library(ggpubr)
library(data.table)
```

1.2 Load datasets

```
#patient cohort
cohort_BAS<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/cohort_BAS.csv")
ursodiol<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/ursodiol.csv")

#metabolomics data
#concentrations
conc_all_filtered<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/conc_all_filte
filtered_combined_table<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/filtered
#annotations
ba_families<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/ba_families.csv")

#16s data
```

```
counts_samples <-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/counts
asv_annotation_blast_ag<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_dataset
asv_alpha_all<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/asv_alph
asv_annotation_blast_color_ag<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_c

#shotgun data
BSH_metalphlan <-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/BSH_me
bai_genes_clean<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/bai_ge
taxa_bas_later<-read_csv("/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/taxa_ba

#ursodiol cohort: double check that it's ok to share
patients_urso_CIF<- read_csv( "/Volumes/vandenbrinklab/Oriana/BAs_published_datasets/pa
```

Chapter 2

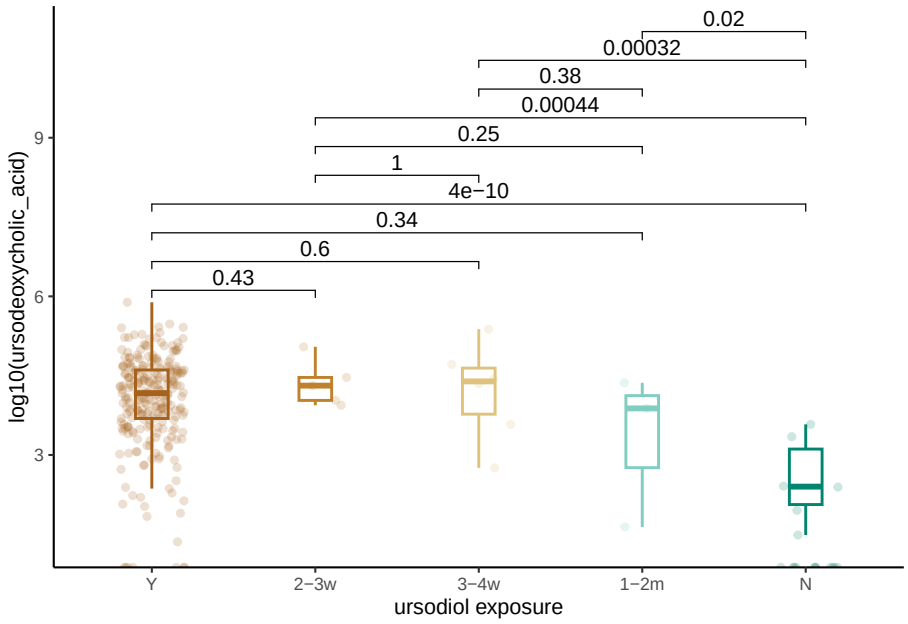
Analyze the effect of UDCA administration on the bile acid pool (supplementary figure 5)

```
#summarize bile acid pools
both_conc<- cohort_BAS %>% select(-ursodiol)%>%
  left_join(conc_all_filtered) %>% clean_names()

#prep dataset prepping each BA depending on its classifications
both_conc_pools<-both_conc %>%
  gather("bile_acid", "value", names(.)[8]:names(.)[ncol(.)]) %>%
  select(-gi_gvhd, -later, -periengr) %>%
  left_join(ba_families) %>%
  filter(bile_acid!="beta_muricholic_acid") %>% #removing because it's not measured in all sample
  filter(bile_acid!="omega_muricholic_acid") %>%  #removing because it's not measured in all samp
  mutate(primary_pool=ifelse(prim_vs_sec=="Primary", value, 0)) %>%
  mutate(secondary_pool=ifelse(prim_vs_sec=="Secondary", value, 0)) %>%
  mutate(sulfated_pool=ifelse(sulfated=="Y", value, 0)) %>%
  mutate(conjugated_pool=ifelse(amidated=="Y" & sulfated=="N", value, 0)) %>%
  mutate(unconjugated_pool=ifelse(amidated=="N" & sulfated=="N", value, 0)) %>%
  mutate(secondary_nonUDCA=ifelse(prim_vs_sec=="Secondary" & udca=="N", value, 0)) %>%
  mutate(total_BAs=value) %>%
  mutate(total_nonUDCA_pool=ifelse(udca=="N", value, 0)) %>%
  mutate(glycine_pool=ifelse(glycine=="Y" & sulfated=="N", value, 0)) %>%
  mutate(taurine_pool=ifelse(taurine=="Y" & sulfated=="N", value, 0)) %>%
```

```
  mutate(taurine_SBA_pool=ifelse(taurine=="Y" & prim_vs_sec=="Secondary" & sulfated=="
  mutate(taurine_PBA_pool=ifelse(taurine=="Y" & prim_vs_sec=="Primary" & sulfated=="N"
  mutate(glycine_SBA_pool=ifelse(glycine=="Y"& prim_vs_sec=="Secondary" & sulfated=="N
  mutate(glycine_PBA_pool=ifelse(glycine=="Y" &prim_vs_sec=="Primary"& sulfated=="N", 
  select(-colnames(ba_families), -value)

both_conc_pools_final<-both_conc_pools %>%
  group_by(sampleid) %>%
  summarise(across(where(is.numeric), sum)) %>%
  left_join(ursodiol)

#rearrange ursodiol
both_conc_pools_final$ursodiol <-factor(both_conc_pools_final$ursodiol,
                            levels=c("Y","2-3w","3-4w","1-2m", "N"))
```

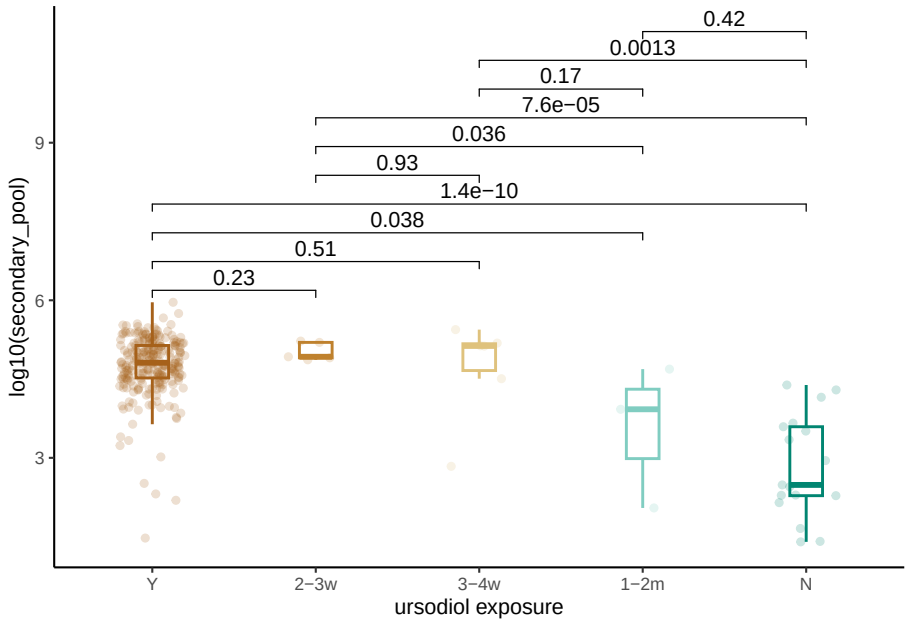
##Evaluation of ursodiol exposure and UDCA concentration

```
ursodiol_BAs<-both_conc %>%
  left_join(ursodiol)

#rearrange ursodiol
ursodiol_BAs$ursodiol <-factor(ursodiol_BAs$ursodiol,
                            levels=c("Y","2-3w","3-4w","1-2m", "N"))

ursodiol_BAs %>%
  ggplot(aes(x=ursodiol, y=log10(`ursodeoxycholic_acid`), color=ursodiol)) +
  geom_boxplot(width=0.2, outlier.shape =NA, lwd=.7)+
  geom_jitter(width=0.2, alpha=0.2)+
  theme_classic() +
  xlab("ursodiol exposure")+
  stat_compare_means(comparisons=list( c("Y", "2-3w"),c("3-4w", "Y"), c("Y", "1-2m"), 
                                       c("3-4w", "2-3w"),c("1-2m", "2-3w"), c("N", "2-
                                       c("1-2m", "3-4w"),  c("N", "3-4w"),
                                       c("N", "1-2m")
  ),
  #label="p.signif",
  method="wilcox.test",
  correct=FALSE)+
  scale_color_manual(values=c("#a6611a", "#bf812d","#dfc27d", "#80cdc1", "#018571"))+
  theme(legend.position="none")
```



2.1 Plot ursodiol exposure and secondary BAs concentrations

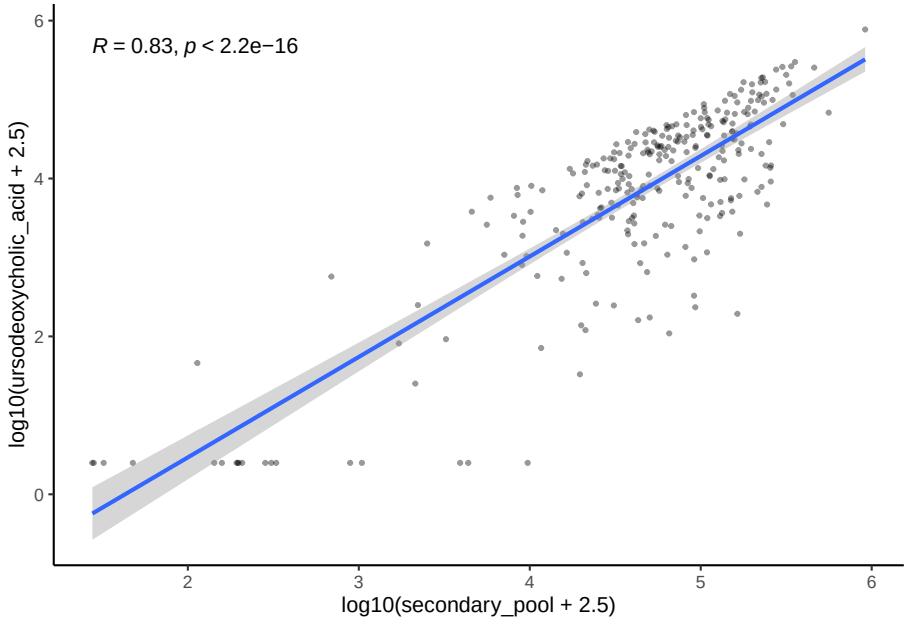
```
both_conc_pools_final %>%
  ggplot(aes(x=ursodiol, y=log10(secondary_pool), color=ursodiol)) +
  geom_boxplot(width=0.2, outlier.shape =NA, lwd=.7)+
  geom_jitter(width=0.2, alpha=0.2)+
  theme_classic() +
  xlab("ursodiol exposure")+
  stat_compare_means(comparisons=list( c("Y", "2-3w"),c("3-4w", "Y"), c("Y", "1-2m"), c("N", "Y"),
                                       c("3-4w", "2-3w"),c("1-2m", "2-3w"), c("N", "2-3w"),
                                       c("1-2m", "3-4w"),  c("N", "3-4w"),
                                       c("N", "1-2m")
  ),
  #label="p.signif",
  method="wilcox.test",
  correct=FALSE)+
  scale_color_manual(values=c("#a6611a", "#bf812d","#dfc27d", "#80cdc1", "#018571"))+
  theme(legend.position="none")
```



2.2 Plot correlation of ursodiol with other bile acid pools: plot conjugated UDCA (tauroursodeoxycholic_acid+glycoursodeoxycholic_acid), TBAs (total_BAs), PBAs (primary_pool), SBAs (secondary_pool), nonUDCA total BAs (total_nonUDCA_pool), nonUDCA SBAs (secondary_nonUDCA), secondary/primary ratio (SP_ratio)

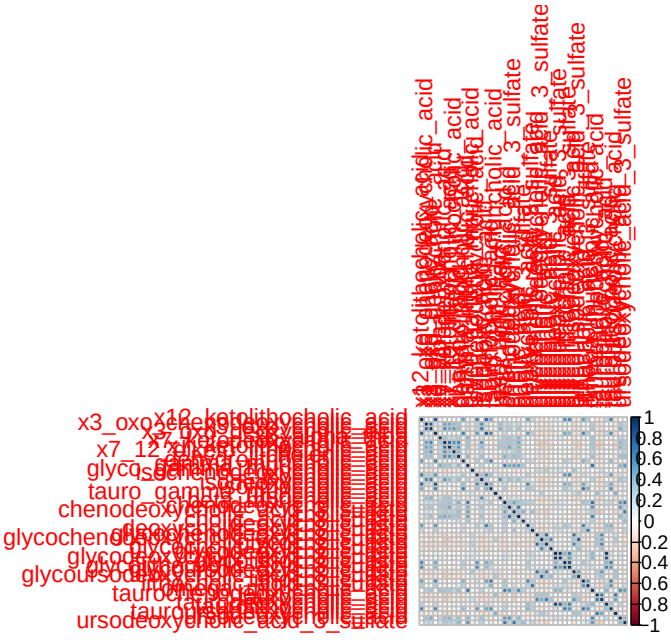
```
both_conc_pools_final %>%
  mutate(SP_ratio=secondary_pool/primary_pool) %>%
  mutate(SP_ratio_nonUDCA=secondary_nonUDCA/primary_pool) %>%
  left_join(both_conc %>% select(sampleid, glycoursodeoxycholic_acid, tauroursodeoxycho
  ggplot(aes(y=log10(`ursodeoxycholic_acid`+2.5), x=log10(secondary_pool+2.5)))+
  geom_point(size=0.8, alpha=0.4)+
  geom_smooth(method="lm")+
  stat_cor(method = "pearson")+
  #ylab("log10(UDCA)")+
```

```
  #xlab("log10(PS_nonUDCA)")+
  theme_classic()
```



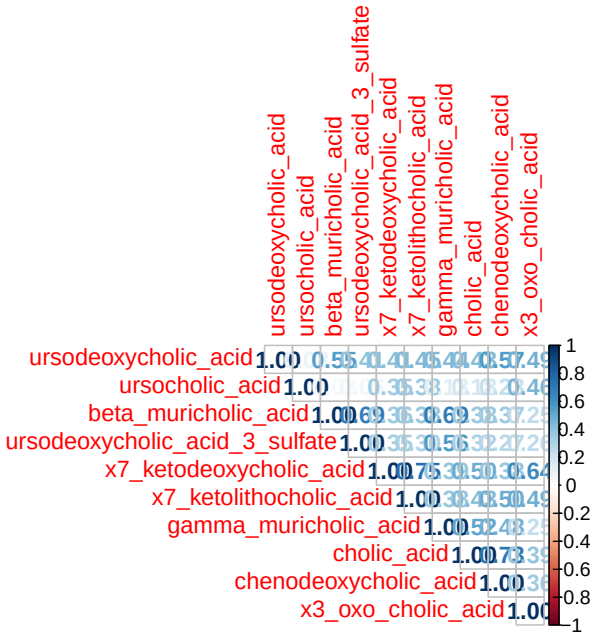
2.3 Create correlation plots to evaluate association of UDCA with all individual BAs

```
library(corrplot)

precor_data<- filtered_combined_table %>%
  column_to_rownames("sampleid")
cor_data<-cor(precor_data, use = "complete.obs")

corrplot(cor_data)
```



2.3.1 Visualization of significant correlations of UDCA with individual BAs (R>0.4)

```
precor_data_selected<- filtered_combined_table %>%
  column_to_rownames("sampleid") %>%
  select(ursodeoxycholic_acid, ursocholic_acid, beta_muricholic_acid, ursodeoxycholic_a
         x7_ketolithocholic_acid, gamma_muricholic_acid, cholic_acid, chenodeoxycholic_

cor_data_selected<-cor(precor_data_selected, use = "complete.obs")

corrplot(cor_data_selected, method = "number", type = "upper")
```


ursodeoxycholic_acid
ursocholic_acid
beta_muricholic_acid
ursodeoxycholic_acid_3_sulfate
x7_ketodeoxycholic_acid
x7_ketolithocholic_acid
gamma_muricholic_acid
cholic_acid
chenodeoxycholic_acid
x3_oxo_cholic_acid
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Chapter 3

Create the bile acid pools (figure 4, supplementary figure 6,8)

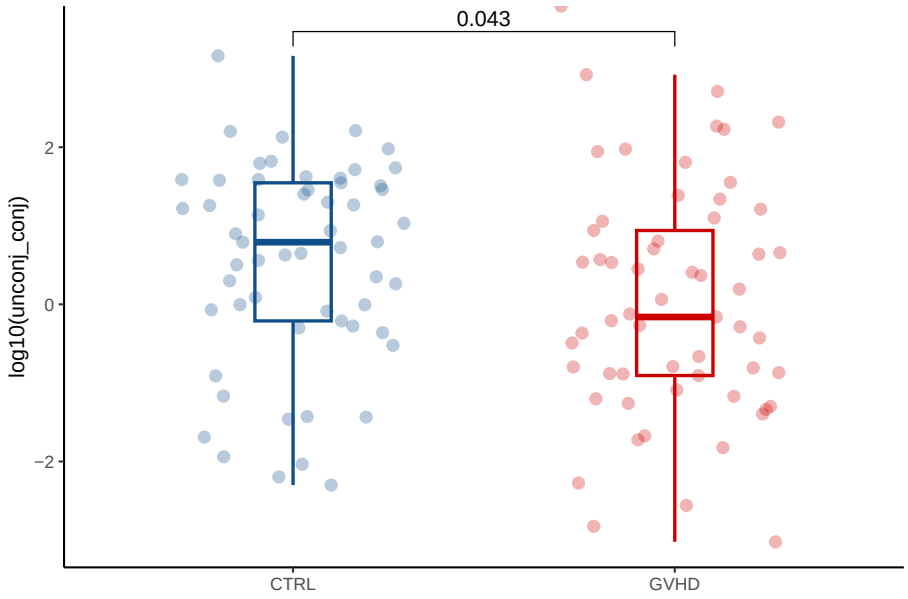
3.1 Create BA pools first for peri-GVHD-onset timepoint

```
later<- cohort_BAS %>%filter(later=="Y") %>%
  filter(ursodiol=="Y") %>%
  select(sampleid, GI_GVHD) %>%
  left_join(conc_all_filtered)

#prep dataset prepping each BA depending on its classifications
later_pools<-later %>%
  gather("bile_acid", "value", names(.)[5]:names(.)[ncol(.)]) %>%
  left_join(ba_families) %>%
  mutate(primary_pool=ifelse(prim_vs_sec=="Primary", value, 0)) %>%
  mutate(secondary_pool=ifelse(prim_vs_sec=="Secondary", value, 0)) %>%
  mutate(sulfated_pool=ifelse(sulfated=="Y", value, 0)) %>%
  mutate(conjugated_pool=ifelse(amidated=="Y" & sulfated=="N", value, 0)) %>%
  mutate(unconjugated_pool=ifelse(amidated=="N" & sulfated=="N", value, 0)) %>%
  mutate(secondary_nonUDCA=ifelse(prim_vs_sec=="Secondary" & udca=="N", value, 0)) %>%
  mutate(total_BAs=value) %>%
  mutate(total_nonUDCA_pool=ifelse(udca=="N", value, 0)) %>%
  mutate(glycine_pool=ifelse(glycine=="Y" & sulfated=="N", value, 0)) %>%
  mutate(taurine_pool=ifelse(taurine=="Y" & sulfated=="N", value, 0)) %>%
```

```
  mutate(taurine_SBA_pool=ifelse(taurine=="Y" & prim_vs_sec=="Secondary" & sulfated=="
  mutate(taurine_PBA_pool=ifelse(taurine=="Y" & prim_vs_sec=="Primary" & sulfated=="N"
  mutate(glycine_SBA_pool=ifelse(glycine=="Y"& prim_vs_sec=="Secondary" & sulfated=="N
  mutate(glycine_PBA_pool=ifelse(glycine=="Y" &prim_vs_sec=="Primary"& sulfated=="N", 
  select(-colnames(ba_families), -value)

#replace NAs with 0 to be able to add sums
later_pools[is.na(later_pools)]<-0

later_pools_final<-later_pools %>%
  #gather("bile_acid", "value", names(.)[2]:names(.)[ncol(.)]) %>%
  #summarise(sum_group=sum(value))
  group_by(sampleid) %>%
  summarise(across(where(is.numeric), sum))
```

3.1.1 Plot: TBAs (total_BAs), PBAs (primary_pool), SBAs (secondary_pool), nonUDCA SBAs (secondary_nonUDCA), conjugated (conjugated_pool), unconjugated (unconjugated_pool), sulfated (sulfated_pool)

```
later_pools_final %>%
  left_join(cohort_BAS) %>%
  mutate(GI_GVHD=ifelse(GI_GVHD=="Y", "GVHD", "CTRL")) %>%
  mutate(sp_ratio=secondary_pool/primary_pool) %>%
  mutate(sp_nonUDCA_ratio=secondary_nonUDCA/primary_pool) %>%
  mutate(unconj_conj=unconjugated_pool/conjugated_pool) %>%
  ggplot(aes(x=GI_GVHD, y=log10(unconj_conj), colour=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(unconj_conj)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("CTRL", "GVHD")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```



3.1.2 Create pies

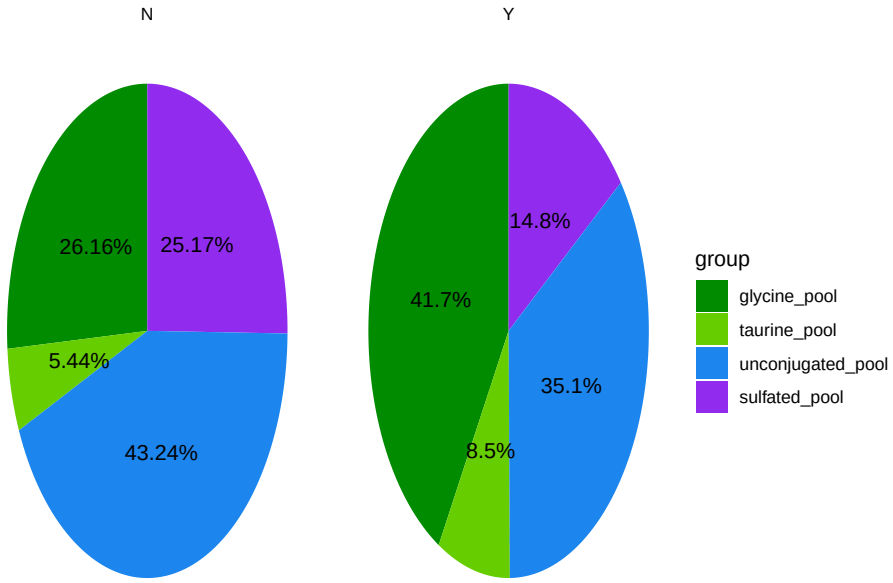
```
dataset_pre<-later_pools_final %>%
  gather("BA_pool", "value", names(.)[2]:names(.)[ncol(.)]) %>%
  left_join(cohort_BAS %>%
              select(sampleid, GI_GVHD, later, ursodiol)) %>%
  filter(later=="Y") %>%
  filter(ursodiol=="Y") %>%
  select(-ursodiol, -later) %>%
  group_by(GI_GVHD, BA_pool) %>%
  summarise(ave_pool=ave(value)) %>% slice(1)
```

```
## Joining with `by = join_by(sampleid)`
```

```
## Warning: Returning more (or less) than 1 row per `summarise()` group was deprecated in
## dplyr 1.1.0.
## i Please use `reframe()` instead.
## i When switching from `summarise()` to `reframe()`, remember that `reframe()`
##   always returns an ungrouped data frame and adjust accordingly.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```
## `summarise()` has grouped output by 'GI_GVHD', 'BA_pool'. You can override
## using the `.groups` argument.
```

```
dataset_pre2<-dataset_pre %>%
  #filter(BA_pool=="primary_pool"|BA_pool=="secondary_nonUDCA") %>% #to evaluate nonUD
  #filter(BA_pool=="primary_pool"|BA_pool=="secondary_pool") %>% #to evaluate total se
  filter(BA_pool=="glycine_pool"|BA_pool=="taurine_pool"|BA_pool=="sulfated_pool"|BA_po
  rename(group=BA_pool) %>%
  rename(value=ave_pool) %>%
  ungroup() %>%
  group_by(GI_GVHD) %>%
  mutate(sum_value=sum(value)) %>%
  mutate(perc=value/sum_value) %>%
  mutate(labels = scales::percent(perc)) %>%
  ungroup()

#only run below when evaluating glycine/taurine conjugation as wel
#define order of piechart for glycine/taurin conjugation
dataset_pre2$group <- factor(dataset_pre2$group, levels = c("glycine_pool", "taurine_po


cp<-coord_polar(theta="y")
cp$is_free<-function()TRUE

ggplot(dataset_pre2, aes(x="", y=perc, fill=group))+
  geom_bar(stat="identity", width=1)+cp+
  facet_wrap(.~GI_GVHD, scales="free")+
  geom_text(aes(label = labels),
            position = position_stack(vjust = 0.5)) +
  theme_void()+
  theme(axis.ticks=element_blank(),
        axis.title=element_blank(),
        axis.text.y=element_blank())+
  scale_fill_manual(values=c("green4","chartreuse3","dodgerblue2","purple2")) #to eval
```



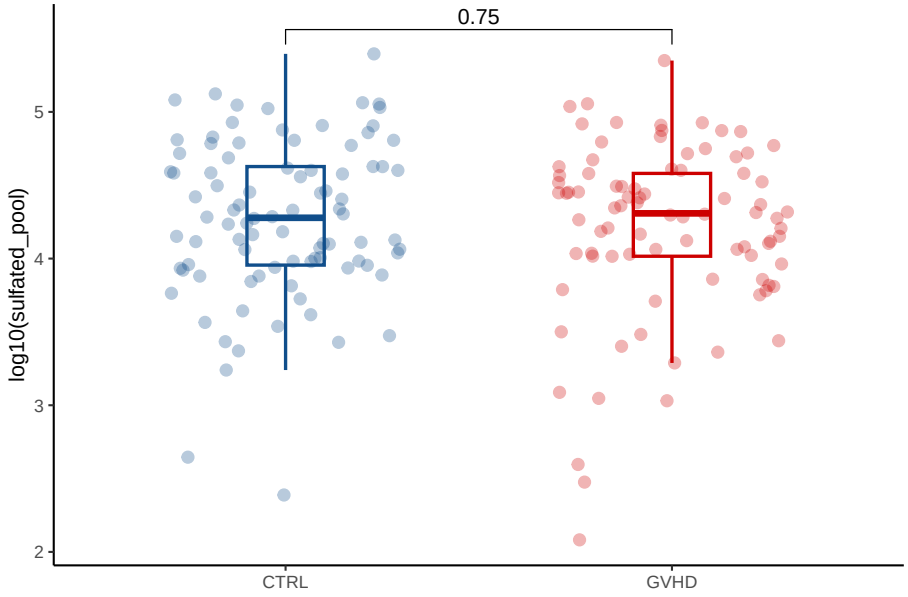
3.2 Create BA pools for peri-engraftment timepoint

```
periengr_conc<- cohort_BAS %>%filter(periengr=="Y") %>%
  filter(ursodiol=="Y") %>%
  select(sampleid, GI_GVHD) %>%
  left_join(conc_all_filtered) %>%
  select(-`beta_muricholic_acid`, -`omega_muricholic_acid`) #remove since it is not measured in c

#prep dataset prepping each BA depending on its classifications
periengr_pools<-periengr_conc %>%
  gather("bile_acid", "value", names(.)[3]:names(.)[ncol(.)]) %>%
  left_join(ba_families) %>%
  mutate(primary_pool=ifelse(prim_vs_sec=="Primary", value, 0)) %>%
  mutate(secondary_pool=ifelse(prim_vs_sec=="Secondary", value, 0)) %>%
  mutate(sulfated_pool=ifelse(sulfated=="Y", value, 0)) %>%
  mutate(conjugated_pool=ifelse(amidated=="Y" & sulfated=="N", value, 0)) %>%
  mutate(unconjugated_pool=ifelse(amidated=="N" & sulfated=="N", value, 0)) %>%
  mutate(secondary_nonUDCA=ifelse(prim_vs_sec=="Secondary" & udca=="N", value, 0)) %>%
  mutate(total_BAs=value) %>%
  mutate(total_nonUDCA_pool=ifelse(udca=="N", value, 0)) %>%
```

```
  mutate(glycine_pool=ifelse(glycine=="Y" & sulfated=="N", value, 0)) %>%
  mutate(taurine_pool=ifelse(taurine=="Y" & sulfated=="N", value, 0)) %>%
  mutate(taurine_SBA_pool=ifelse(taurine=="Y" & prim_vs_sec=="Secondary" & sulfated=="
  mutate(taurine_PBA_pool=ifelse(taurine=="Y" & prim_vs_sec=="Primary" & sulfated=="N"
  mutate(glycine_SBA_pool=ifelse(glycine=="Y"& prim_vs_sec=="Secondary" & sulfated=="N
  mutate(glycine_PBA_pool=ifelse(glycine=="Y" &prim_vs_sec=="Primary"& sulfated=="N",
  select(-colnames(ba_families), -value)

#replace NAs with 0 to be able to add sums
periengr_pools[is.na(periengr_pools)]<-0

#final table with each group sum
periengr_pools_final<-periengr_pools %>%
  group_by(sampleid) %>%
  summarise(across(where(is.numeric), sum))
```

3.2.1 Plot BA pools and GVHD; can plot total BAs (total_BAs), PBAs (primary_pool), SBAs (secondary_pool), nonUDCA SBAs (secondary_nonUDCA), conjugated (conjugated_pool), unconjugated (unconjugated_pool), sulfated_pool, secondary/primary ratio and secondary*/primary ratio

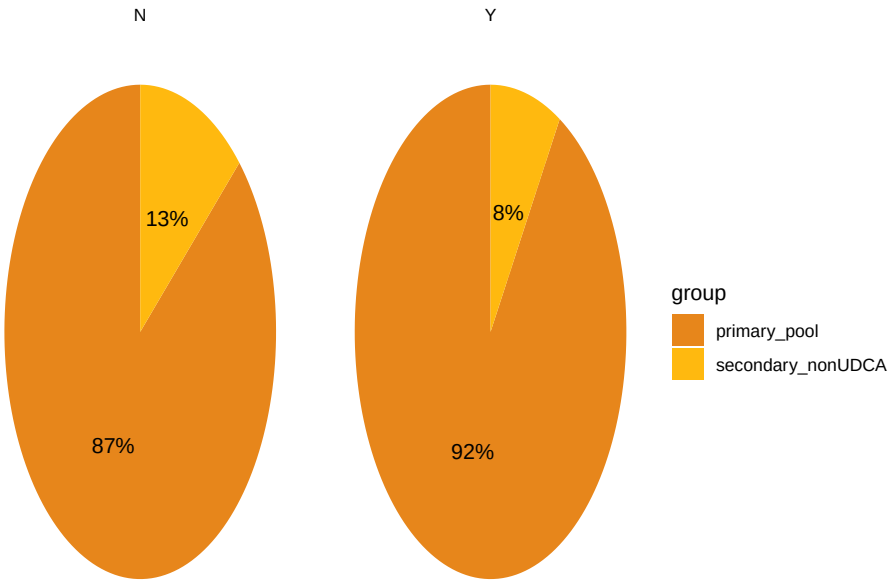
```
periengr_pools_final %>%
  left_join(cohort_BAS) %>%
  mutate(GI_GVHD=ifelse(GI_GVHD=="Y", "GVHD", "CTRL"))  %>%
  mutate(sp_ratio=secondary_pool/primary_pool) %>%
  mutate(sp_nonUDCA_ratio=secondary_nonUDCA/primary_pool) %>%
  ggplot(aes(x=GI_GVHD, y=log10(sulfated_pool), colour=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  #ylab("log10(sulfated_pool)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("CTRL", "GVHD")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```



3.2.2 Create pies

```
dataset_pre<-periengr_pools_final %>%
  gather("BA_pool", "value", names(.)[2]:names(.)[ncol(.)]) %>%
  left_join(cohort_BAS %>% select(sampleid, GI_GVHD, ursodiol)) %>%
  filter(ursodiol=="Y") %>%
  group_by(GI_GVHD, BA_pool) %>%
  summarise(ave_pool=ave(value)) %>% slice(1)

dataset_pre2<-dataset_pre %>%
  filter(BA_pool=="primary_pool"|BA_pool=="secondary_nonUDCA") %>% #to evaluate nonUDCA secondary
  #filter(BA_pool=="primary_pool"|BA_pool=="secondary_pool") %>% #to evaluate total secondary
  #filter(BA_pool=="glycine_pool"|BA_pool=="taurine_pool"|BA_pool=="sulfated_pool"|BA_pool=="unco
  rename(group=BA_pool) %>%
  rename(value=ave_pool) %>%
  ungroup() %>%
  group_by(GI_GVHD) %>%
  mutate(sum_value=sum(value)) %>%
  mutate(perc=value/sum_value) %>%
  mutate(labels = scales::percent(perc)) %>%
  ungroup()

#only run to evaluate glycine/taurine conjugation
```

```
#define order of piechart for glycine/taurin conjugation
#dataset_pre2$group <- factor(dataset_pre2$group, levels = c("glycine_pool", "taurine_

cp<-coord_polar(theta="y")
cp$is_free<-function()TRUE

ggplot(dataset_pre2, aes(x="", y=perc, fill=group))+
  geom_bar(stat="identity", width=1)+cp+
  facet_wrap(.~GI_GVHD, scales="free")+
  geom_text(aes(label = labels),
            position = position_stack(vjust = 0.5)) +
  theme_void()+
  theme(axis.ticks=element_blank(),
        axis.title=element_blank(),
        axis.text.y=element_blank())+
  #scale_fill_manual(values=c("green4","chartreuse3","dodgerblue2","purple2"))
  scale_fill_manual(values=c("#E7861B","darkgoldenrod1")) #for primary/secondary
```

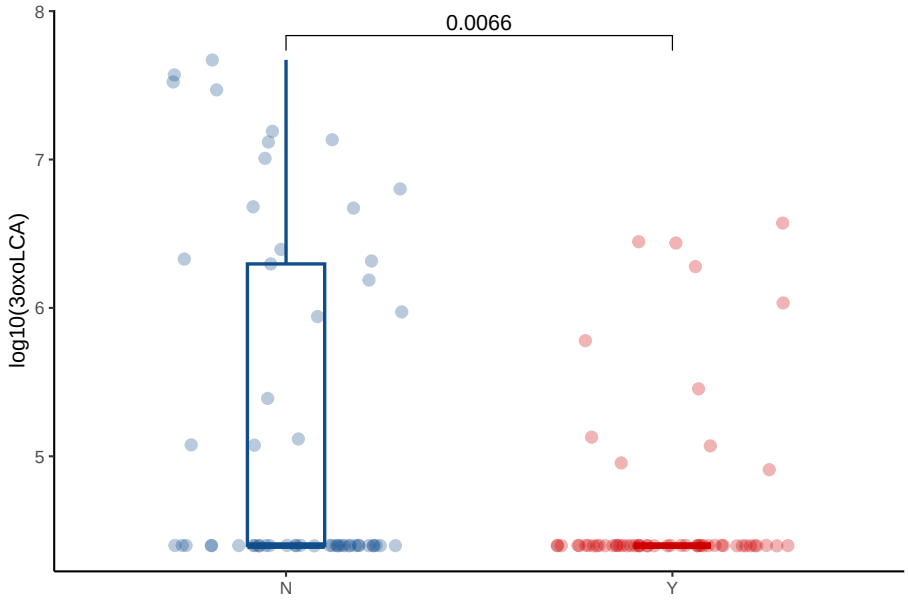


Chapter 4

Evaluate T cell modulatory BAs in patients with GVHD vs controls (figure 4)

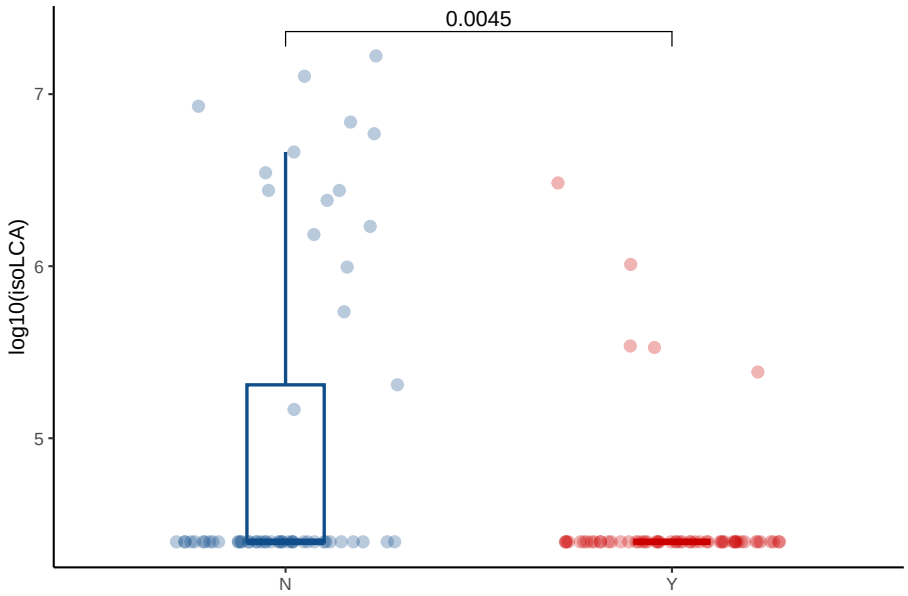
4.1 3oxoLCA

```
filtered_combined_table %>%
  left_join(cohort_BAS) %>%
  filter(later=="Y") %>%
  filter(ursodiol=="Y") %>%
  ggplot(aes(y=log10(dehydrolithocholic_acid+25000), x=GI_GVHD, color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(3oxoLCA)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```



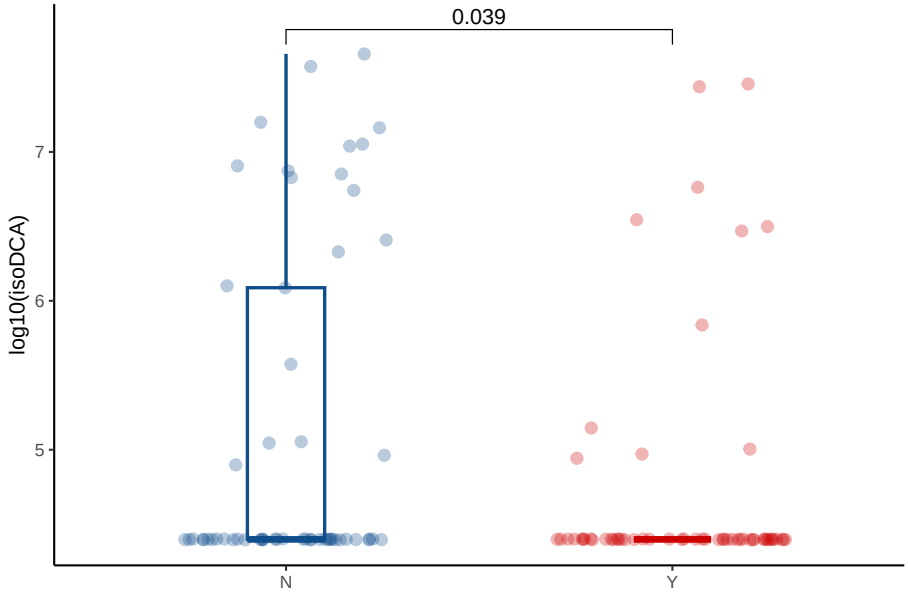
4.2 isoLCA

```
filtered_combined_table %>%
  left_join(cohort_BAS) %>%
  filter(later=="Y") %>%
  filter(ursodiol=="Y") %>%
  ggplot(aes(y=log10(isolithocholic_acid+25000), x=GI_GVHD, color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(isoLCA)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```



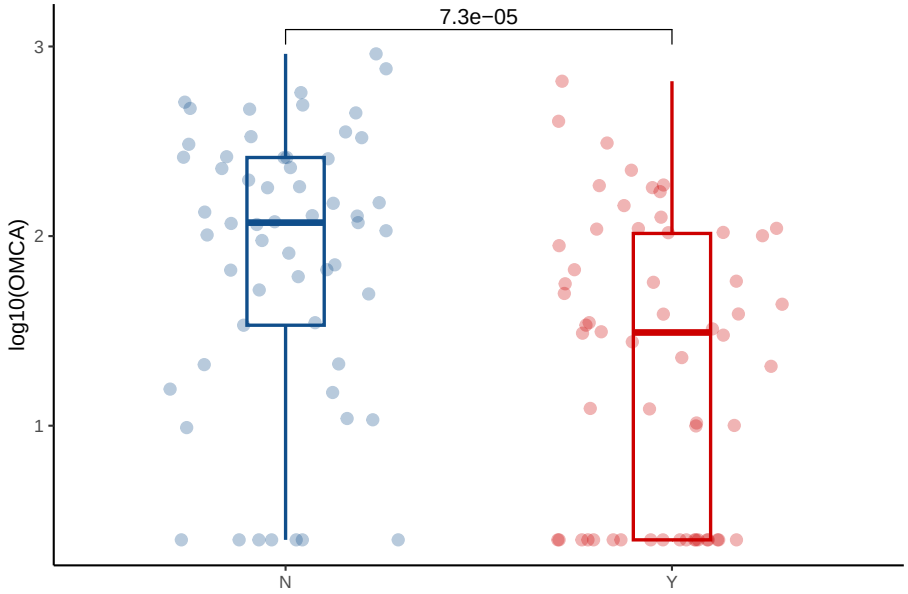
4.3 isoDCA

```
filtered_combined_table %>%
  left_join(cohort_BAS) %>%
  filter(later=="Y") %>%
  filter(ursodiol=="Y") %>%
  ggplot(aes(y=log10(isodeoxycholic_acid+25000), x=GI_GVHD, color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(isoDCA)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```



4.4 OMCA

```
conc_all_filtered %>%
  left_join(cohort_BAS) %>%
  filter(later=="Y") %>%
  filter(ursodiol=="Y") %>%
  ggplot(aes(y=log10(omega_muricholic_acid+2.5), x=GI_GVHD, color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(OMCA)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```


7.3e−05
log10(OMCA)
3
2
1
N
Y

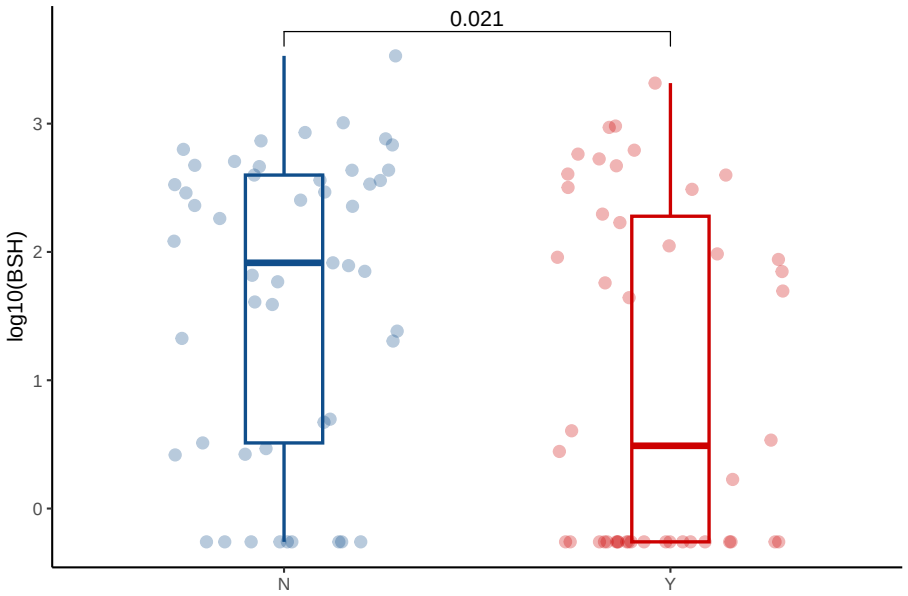
Chapter 5

Shotgun metagenomic sequencing: Evaluate genes of interest (figure 5, supplement figure 10)

5.1 BSH

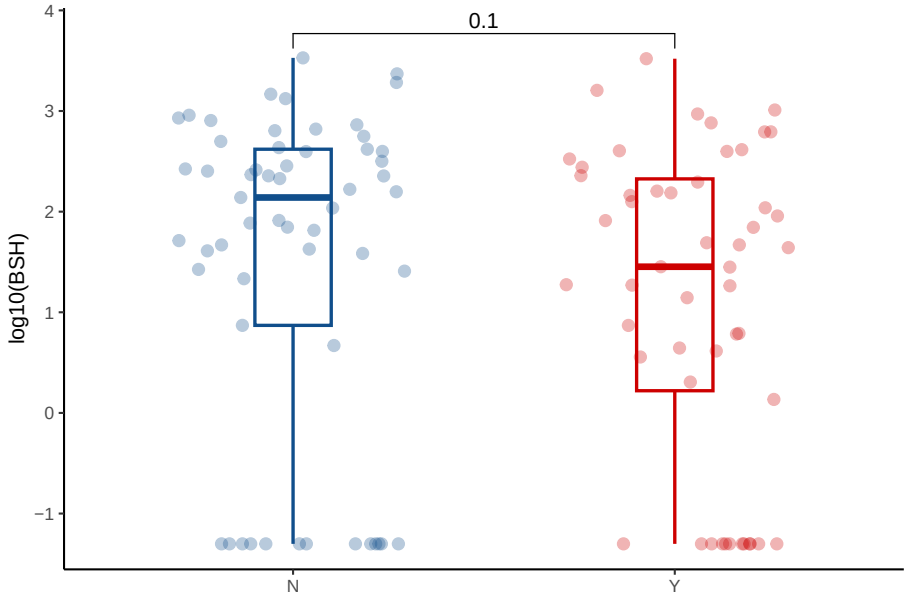
5.1.1 Evaluate BSH abundance at peri-GVHD onset

```
BSH_metalphlan %>%
  left_join(cohort_BAS) %>%
  filter(later=="Y") %>%
  ggplot(aes(x=GI_GVHD, y=log10(cpm+0.55), color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(BSH)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```



5.1.2 Evaluate BSH abundance at peri-engraftment time point

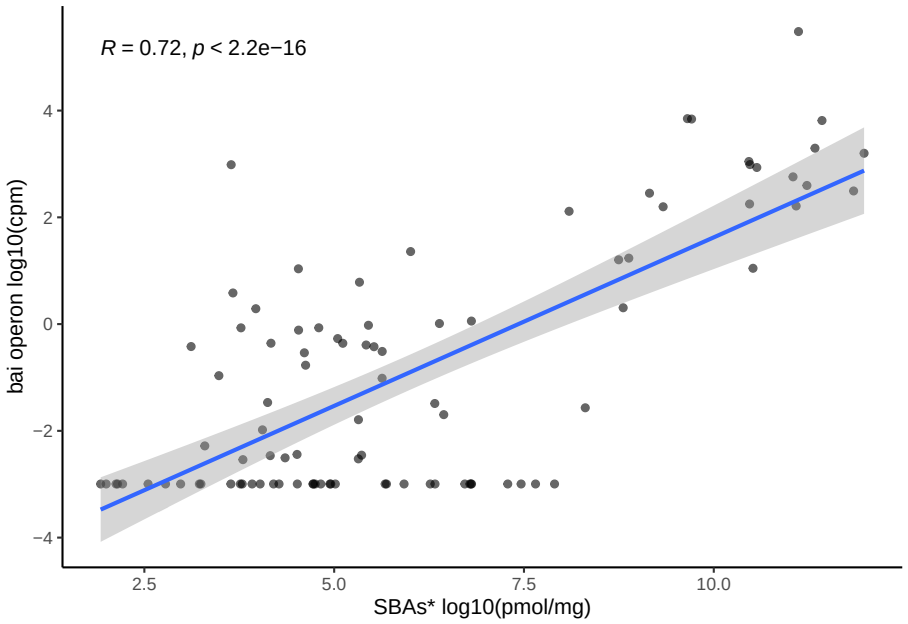
```
BSH_metalphlan %>%
  left_join(cohort_BAS) %>%
  filter(periengr=="Y") %>%
  ggplot(aes(x=GI_GVHD, y=log10(cpm+0.05), color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(BSH)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```



5.2 Bai operon gene

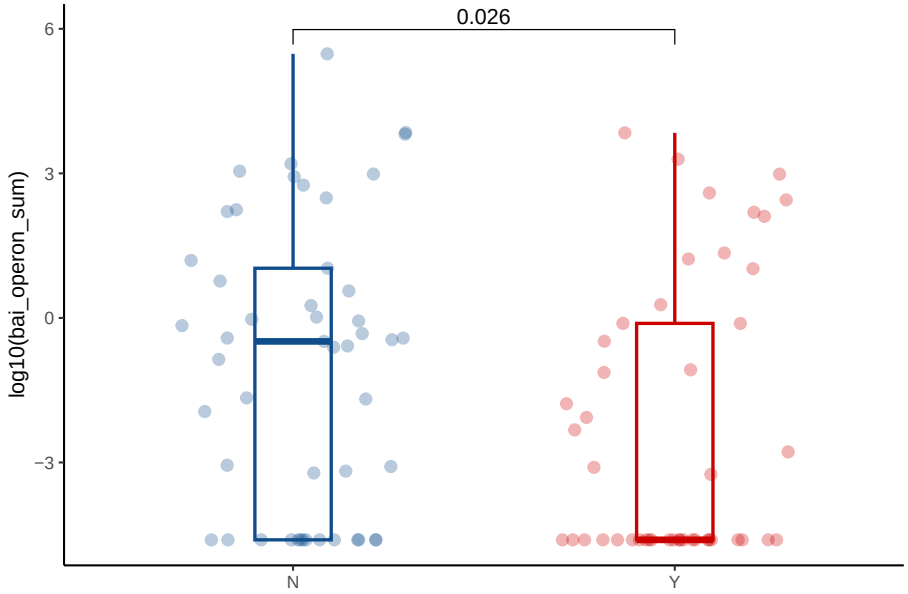
5.2.1 Evaluate correlation of bai operon gene sum and nonUDCA secondary BAs

```
bai_genes_clean %>%
  distinct(sampleid, bai_operon_sum ) %>%
  inner_join(both_conc_pools_final) %>%
  ggplot(aes(x=log(secondary_nonUDCA), y=log(bai_operon_sum+0.05)))+
  geom_point(alpha=0.6)+
  stat_cor(method="pearson")+
  geom_smooth(method="lm")+
  theme_classic()+
  ylab("bai operon log10(cpm)")+
  xlab("SBAs* log10(pmol/mg)")
```



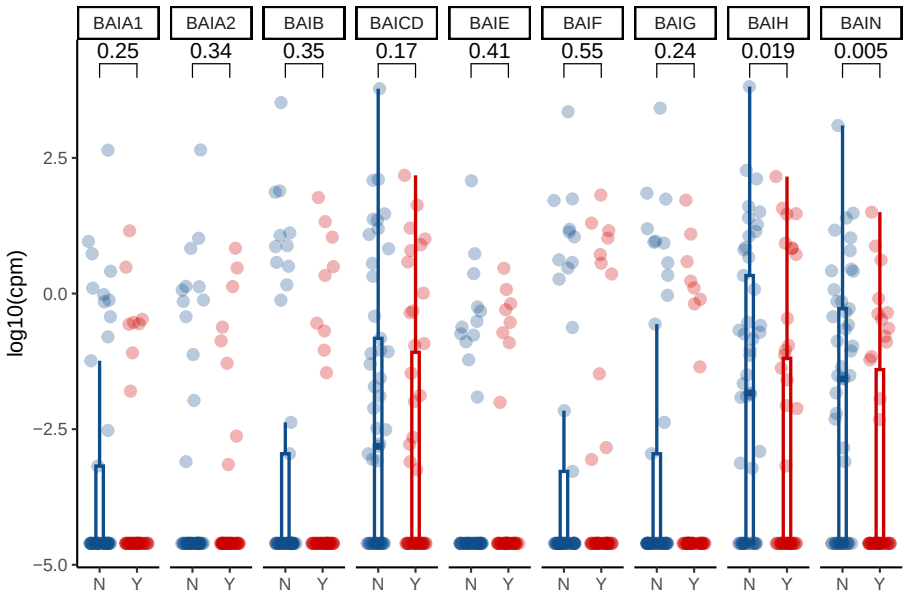
5.2.2 Bai operon gene sum in peri-GVHD onset

```
bai_genes_clean %>%
  distinct(sampleid, bai_operon_sum ) %>%
  inner_join(cohort_BAS %>% select(sampleid, GI_GVHD, later) %>% filter(later=="Y")) %>
  ggplot(aes(x=GI_GVHD, y=log(bai_operon_sum+0.01), color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5) +
  ylab("log10(bai_operon_sum)") +
  xlab("") +
  theme_classic() +
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3")) +
  theme(legend.position="none")
```



5.2.3 Bai operon individual gene abundance

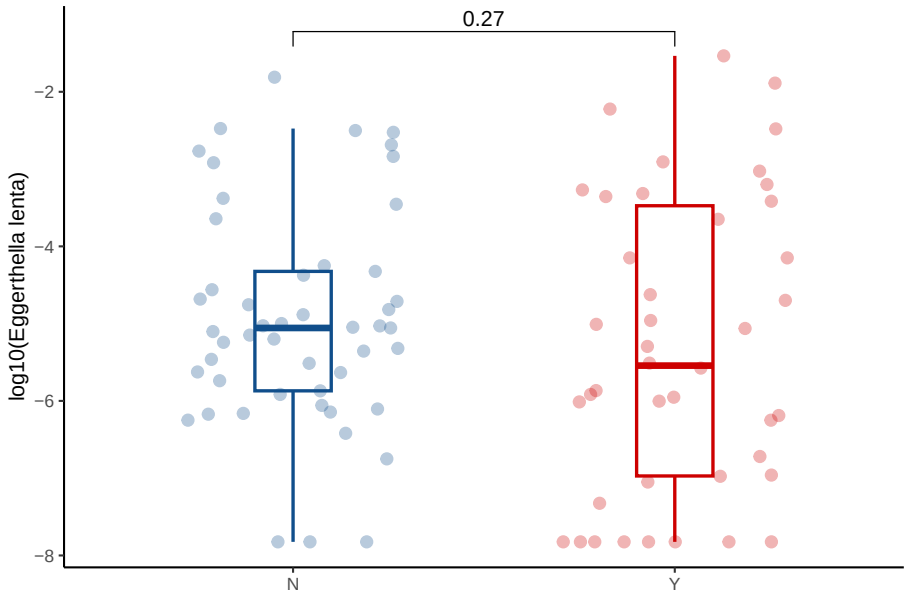
```
bai_genes_clean %>%
  inner_join(cohort_BAS %>% select(sampleid, GI_GVHD, later) %>% filter(later=="Y")) %>%
  ggplot(aes(x=GI_GVHD, y=log(cpm+0.01), color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(cpm)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")+
  facet_grid(.~gene)
```



5.3 Bile acid related bacteria

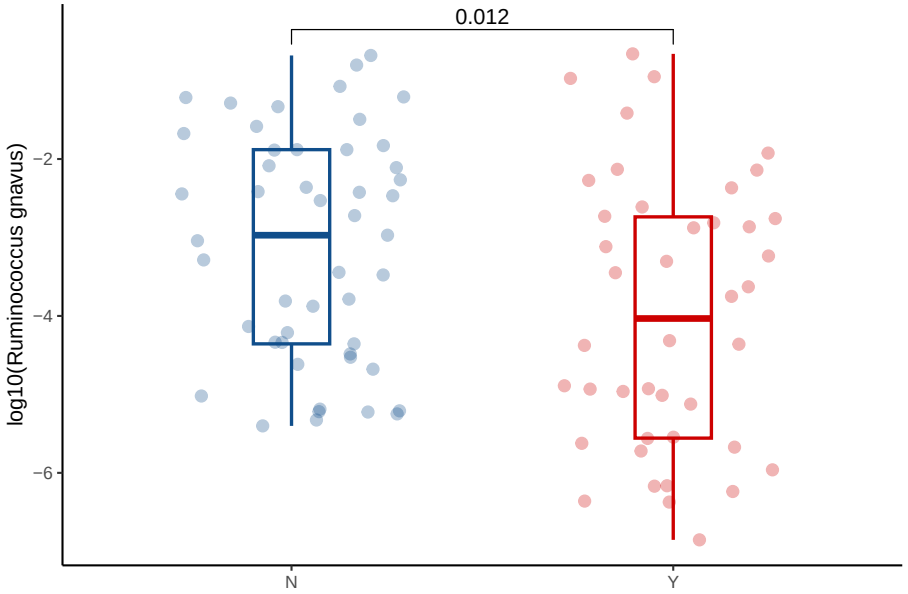
5.3.1 Eggerthella lenta

```
taxa_bas_later %>%
  ggplot(aes(x=GI_GVHD, y=log10(eggerthella_lenta+ 1.5e-08), color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(Eggerthella lenta)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("N", "Y")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```



5.3.2 Ruminococcus gnavus

```
taxa_bas_later %>%
  ggplot(aes(x=GI_GVHD, y=log10(ruminococcus_gnavus+ 1.4e-07), color=GI_GVHD))+
  geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(Ruminococcus gnavus)")+
  xlab("")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("N", "Y")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("dodgerblue4", "red3"))+
  theme(legend.position="none")
```


0.012
log10(Ruminococcus gnavus)
−2
−4
−6
N
Y

Chapter 6

Create landscape of all peri-GVHD-onset samples (figure 5)

6.1 Define sample order from higher nonUDCA secondary BAs to lower

```
samples_key<-BSH_metalphlan %>% distinct(sampleid) %>%
  left_join(later_pools_final %>% select(sampleid, secondary_nonUDCA)) %>%
  left_join(cohort_BAS) %>%
  filter(later=="Y") %>%
  arrange(desc(secondary_nonUDCA)) %>%
  left_join(ursodiol) %>% filter(ursodiol2=="Y")

level_order <- samples_key$sampleid
```

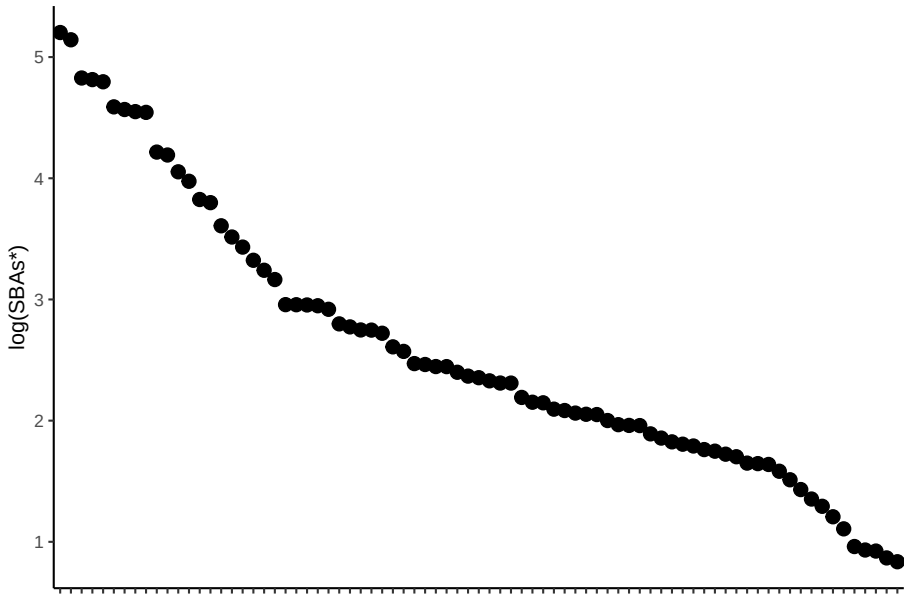
6.2 GI GVHD plot

```
gi_gvhd_plot<-cohort_BAS %>%
  filter(later=="Y") %>%
  ggplot((aes(x = factor(sampleid, levels = level_order), y = 1, fill = GI_GVHD))) +
  geom_raster(color = "black", size = 0.5) +
  theme_classic()+ theme(axis.text.x=element_blank())+
```

```
  xlab("")+
  ylab("")+
  scale_fill_manual(values=c("white", "dodgerblue4"))+
  theme(axis.text.y = element_blank())+
  theme(legend.position = "none") #only for plotting reasons

gi_gvhd_plot
```

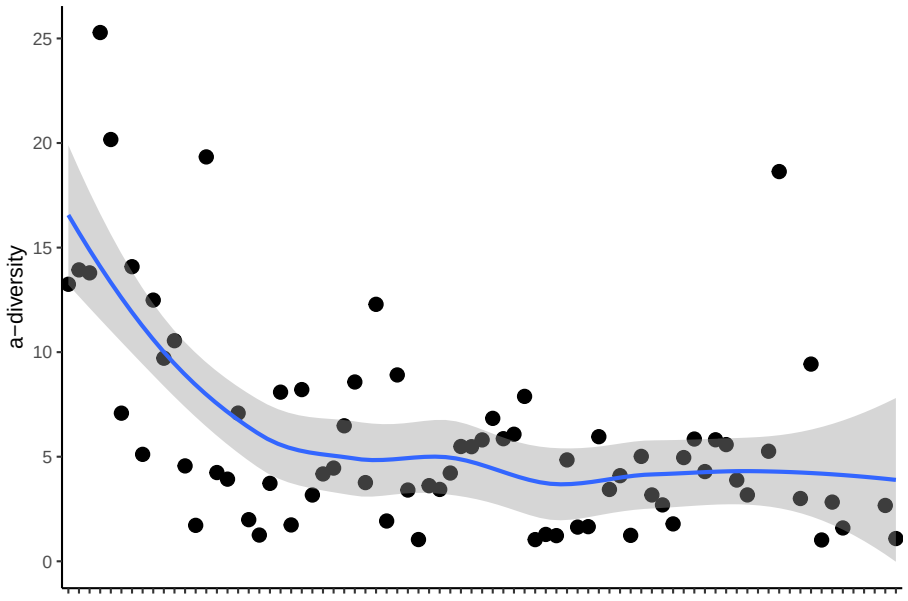
6.3 SBA plot

```
sba_plot<-ggplot(samples_key, aes(x=factor(sampleid, level=level_order), y=log10(secon
  geom_point(size=3)+theme_classic()+
  ylab("log(SBAs*)")+
  theme(axis.text.x=element_blank())+
  xlab("")
sba_plot
```



6.4 A-diversity plot

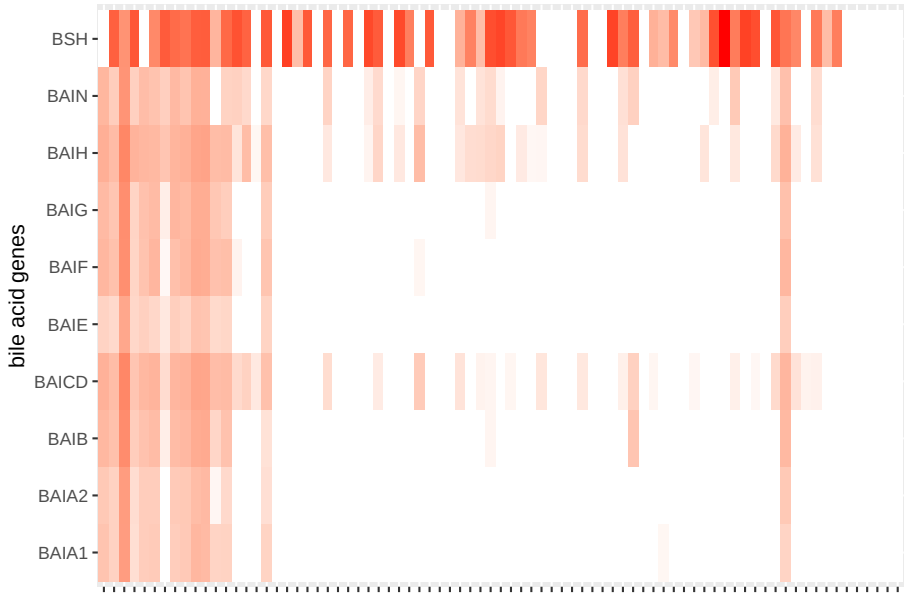
```
adiv_pre<-cohort_BAS %>%
  filter(later=="Y") %>%
  left_join(asv_alpha_all) %>%  #add a-diversity
  inner_join(samples_key) %>%
  arrange(desc(secondary_nonUDCA)) %>%
          mutate(rank = 1:nrow(.))

adiv_plot<-ggplot(adiv_pre, aes(x = rank, y = simpson_reciprocal)) +
  geom_point(size=3) +
  geom_smooth(method = "loess") +
  theme_classic() +
  ylab("a-diversity") +
  #xlab("sampleid") +
  theme(axis.text.x = element_blank()) +
  xlab("") +
  scale_x_discrete(limits = adiv_pre$rank[order(-adiv_pre$rank)])

adiv_plot
```



6.5 Bile acid related genes

```
bai_genes_clean$sampleid <-gsub("FMT_", "FMT.", bai_genes_clean$sampleid)

ba_genes_pre<-samples_key %>%
  select(sampleid, GI_GVHD, secondary_nonUDCA) %>%
  left_join(BSH_metalphlan %>%
              select(sampleid, cpm, KOID)) %>%
  rename(gene=KOID) %>%
  mutate(gene=ifelse(gene=="K01442", "BSH", NA)) %>%
  distinct() %>%
  spread(key=gene, value=cpm, fill=0)

operon_genes_pre<-samples_key %>%
  left_join(bai_genes_clean) %>%
  select(sampleid, cpm, gene) %>%
  distinct() %>%
  spread(key=gene, value=cpm, fill=0)

pre_bai_plot<-ba_genes_pre %>%
  left_join(operon_genes_pre) %>%
  select(-GI_GVHD, -secondary_nonUDCA) %>%
```

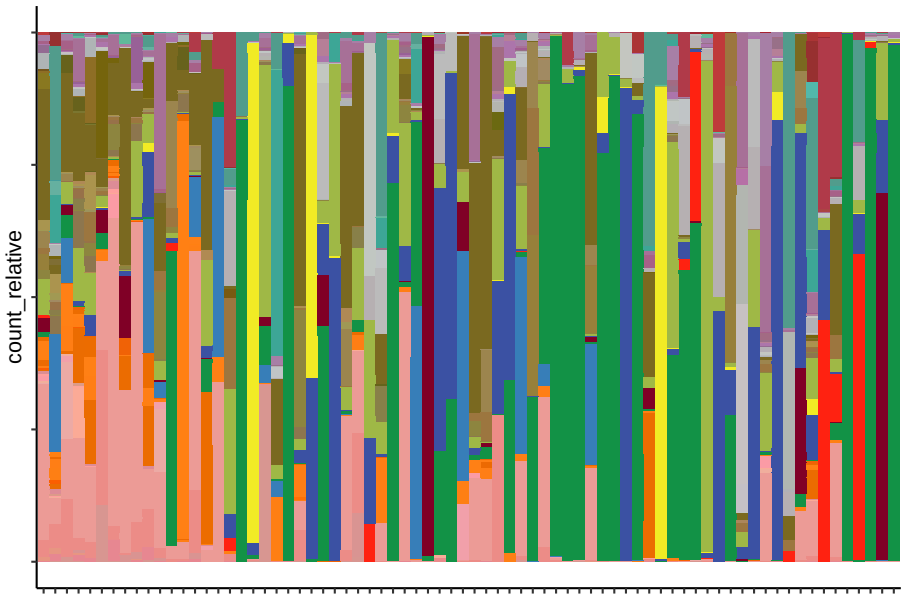
```
  gather("gene", "cpm", names(.)[2]:names(.)[ncol(.)])


bai_plot<-pre_bai_plot %>%
  ggplot(aes(x=factor(sampleid, level=level_order), y=gene, fill=log10(cpm+0.05)))+
           geom_tile()+
  xlab("")+
  ylab("bile acid genes")+
  scale_fill_gradient(low="white", high="red")+
  theme(axis.text.x=element_blank())+
  theme(legend.position = "none") #only for plotting reasons
bai_plot
```



6.6 Microbiome composition

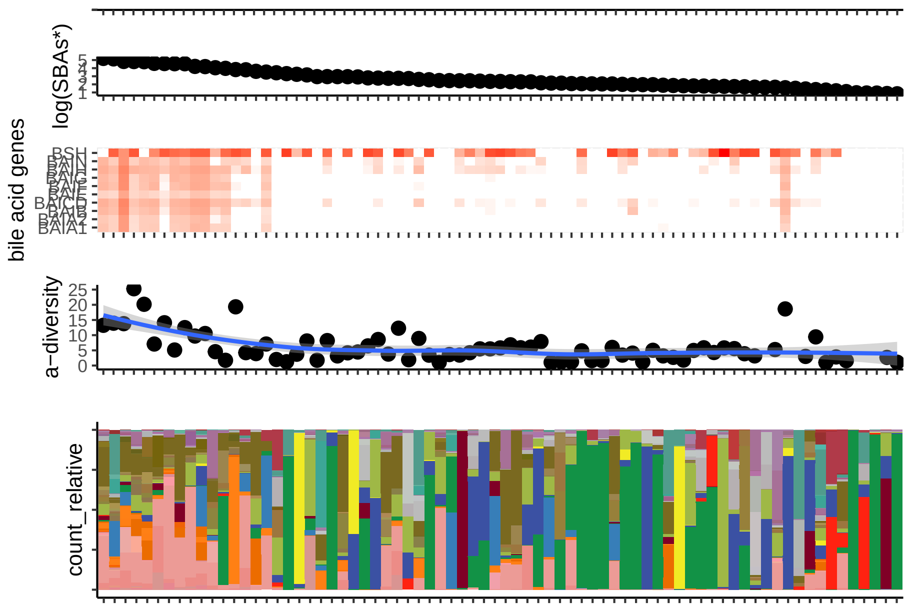
```
setDT(asv_annotation_blast_color_ag)
asv_color_base_set = unique(asv_annotation_blast_color_ag[,.(color_label_group,color_base)])
color_base_set_asv_carT = asv_color_base_set$color_base
names(color_base_set_asv_carT) =asv_color_base_set$color_label_group;
gg = ggplot(asv_color_base_set, aes(color_label_group,y=1,fill=color_label_group)) + geom_tile()
  scale_fill_manual(values = color_base_set_asv_carT) +
```

```
  theme_classic() +
  theme(axis.text.x = element_text(angle=60,hjust = 1)) +
  theme(legend.position = "none")

#color_set_asv_carT maps each distinct taxonomic group to its corresponding color.
asv_color_set = unique(asv_annotation_blast_color_ag[,.(color,color_label_group_distinc
color_set_asv_carT = asv_color_set$color
names(color_set_asv_carT) =asv_color_set$color_label_group_distinct;
setDT(counts_samples)
setDT(asv_annotation_blast_color_ag)
m = merge(counts_samples[,.(asv_key,sampleid,
                            count,count_relative,count_total)],
          asv_annotation_blast_color_ag[,.(asv_key,color_label_group_distinct)]);

sample_composition <- m %>%
  left_join(cohort_BAS %>% select(PID, sampleid)) %>%
  left_join(cohort_BAS) %>%
  filter(later=="Y")


m1<-sample_composition %>%
  group_by(sampleid, color_label_group_distinct) %>%
  inner_join(samples_key) %>%
  mutate(sampleid = fct_reorder(sampleid, desc(secondary_nonUDCA)))

m1$color_label_group_distinct = factor(m1$color_label_group_distinct,levels = sort(unic
gg_composition = ggplot(m1,
                        aes(x=factor(sampleid, levels=level_order),
                            y=count_relative,
                            fill=color_label_group_distinct) ) +
  geom_bar(stat = "identity",position="fill",width = 1) +
  theme_classic() +
  theme(axis.text.x = element_blank(),
        axis.text.y = element_blank(),
        legend.position = "none") +
  xlab("")+
  scale_fill_manual(values = color_set_asv_carT);
print(gg_composition)
```



6.7 Add all plots together

```
library(cowplot)
last<-plot_grid(gi_gvhd_plot, sba_plot, bai_plot, adiv_plot, gg_composition,
          #labels = c("A", "B", "C", "D", "E"),
          ncol = 1, nrow = 5,
          align = "v",axis = 'l',
          width = 40, height = 20,
          rel_heights = c(1.8, 4 ,6, 6, 10))
last
```

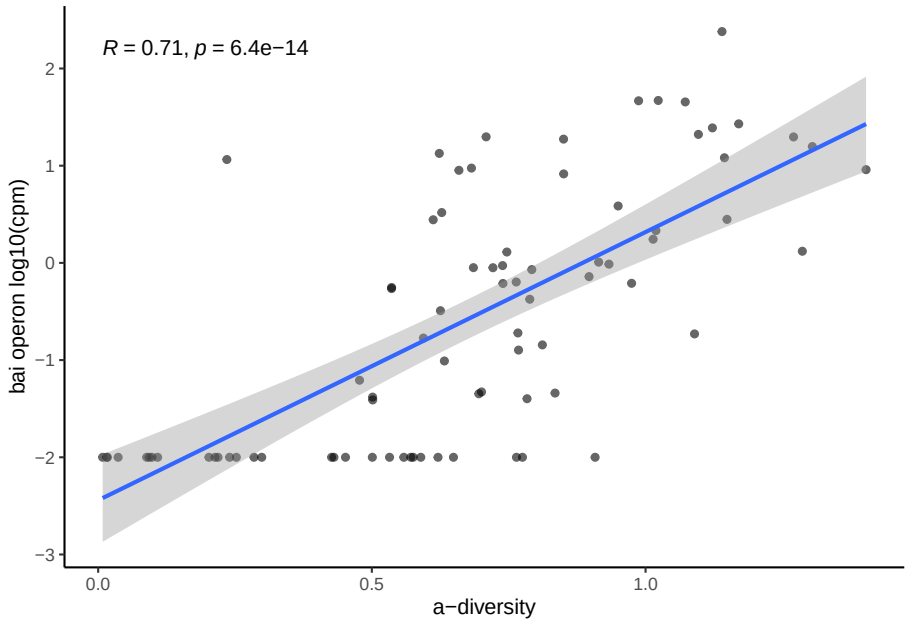

log(SBAs*)
5
4
3
2
1
bile acid genes
BSH
a−diversity
25
20
15
10
5
0
count_relative

Chapter 7

Diversity, bai operon and domination

7.1 Evaluate correlation of a-diversity and bai operon sum

```
data_ba<- asv_alpha_all %>%
  inner_join(bai_genes_clean %>% distinct(sampleid, bai_operon_sum)) %>%
  left_join(cohort_BAS) %>%
  filter(ursodiol=="Y")

data_ba %>%
ggplot(aes(x=log10(simpson_reciprocal), y=log10(bai_operon_sum+0.01)))+
  geom_point(alpha=0.6)+
  stat_cor(method="pearson")+
  geom_smooth(method="lm")+
  theme_classic()+
  ylab("bai operon log10(cpm)")+
  xlab("a-diversity")
```



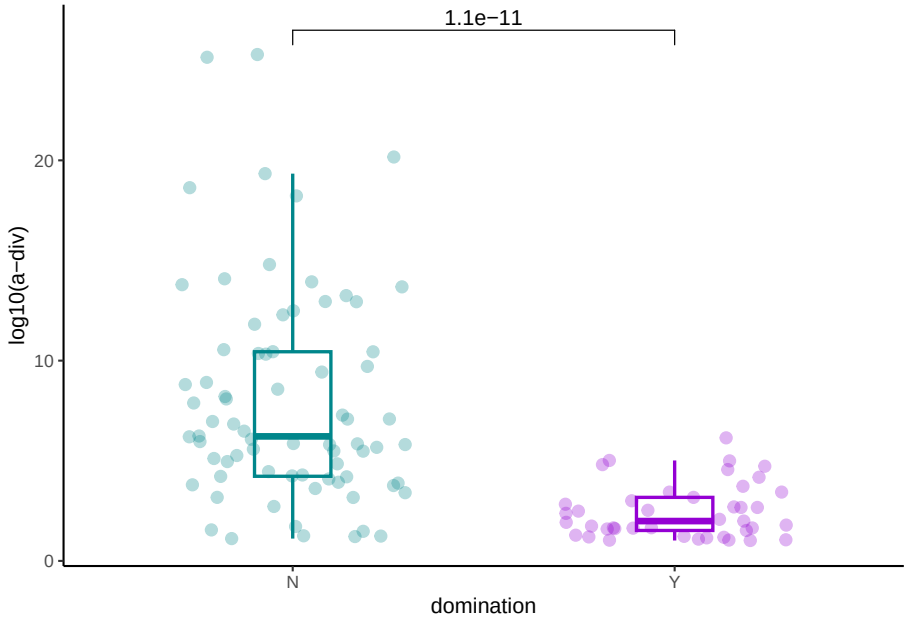
7.2 Identify patients with monodomination by 16S

```
#create dataset with asv
samples_asv<-cohort_BAS %>%
  filter(later=="Y") %>%
  select(sampleid) %>%
  inner_join(counts_samples %>%
               select(sampleid, asv_key, count, count_total)) %>%
  inner_join(asv_annotation_blast_ag %>%
               select(asv_key, kingdom, phylum, class, ordr, family, genus)) %>%
  mutate(relab=count/count_total) %>%
  group_by(sampleid, genus)

pathogens_pre<- samples_asv %>%
  filter(genus=="Enterococcus"|genus=="Streptococcus"|phylum=="Proteobacteria") %>%
  mutate(enterococcus=ifelse(genus=="Enterococcus", relab, 0)) %>%
  mutate(streptococcus=ifelse(genus=="Streptococcus", relab, 0)) %>%
  mutate(proteobacteria=ifelse(phylum=="Proteobacteria", relab, 0)) %>%
  mutate(enterococcus_dom=ifelse(enterococcus>=0.3, "Y", "N")) %>%
  mutate(streptococcus_dom=ifelse(streptococcus>=0.3, "Y", "N")) %>%
   mutate(proteobacteria_dom=ifelse(proteobacteria>=0.3, "Y", "N")) %>%
```

```
  mutate(any_dom=ifelse(enterococcus_dom=="Y"|streptococcus_dom=="Y"|proteobacteria_dom=="Y", "Y"
  group_by(sampleid) %>%
  arrange(desc(any_dom)) %>% slice(1)
```

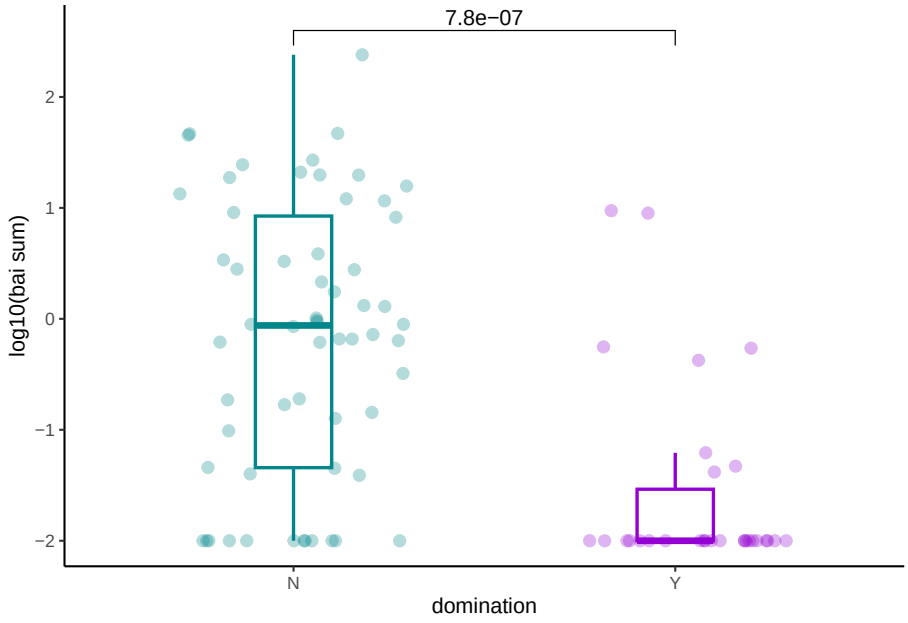
7.3 Domination and a-diversity

```
pathogens_pre %>% inner_join(asv_alpha_all) %>%
  ggplot(aes(x=any_dom, y=simpson_reciprocal, color=any_dom))+
   geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(a-div)")+
  xlab("domination")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("turquoise4","darkviolet"))+
  theme(legend.position="none")
```



7.4 Domination and bai operon

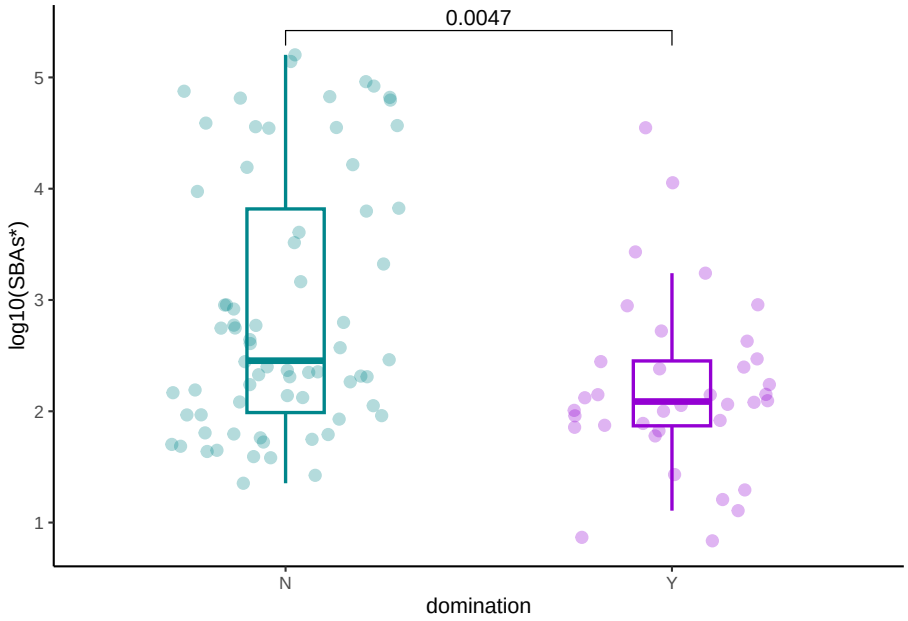
```
pathogens_pre %>% inner_join(bai_genes_clean %>% distinct(sampleid, bai_operon_sum)) %>
  ggplot(aes(x=any_dom, y=log10(bai_operon_sum+0.01),  color=any_dom))+
   geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(bai sum)")+
  xlab("domination")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("turquoise4","darkviolet"))+
  theme(legend.position="none")
```



7.5 SBAs and domination

```
pathogens_pre %>% inner_join(later_pools_final) %>%
  ggplot(aes(x=any_dom, y=log10(secondary_nonUDCA), color=any_dom))+
   geom_boxplot(width=0.2, lwd=0.8, outlier.shape = NA) +
```

```
  geom_jitter(width=0.3, alpha=0.3, size=2.5)+
  ylab("log10(SBAs*)")+
  xlab("domination")+
  theme_classic()+
  stat_compare_means(comparisons=list(c("Y", "N")),
                     method="wilcox.test",
                     correct=FALSE)+
  scale_color_manual(values=c("turquoise4","darkviolet"))+
  theme(legend.position="none")
```



Chapter 8

Evaluation of UDCA exposure and clinical outcomes

8.1 Prepare the patient outcome table

```
library(tidycmprsk)
library(ggsurvfit)
library(tidycmprsk)
library(gtsummary)

patients_urso_CIF2<-patients_urso_CIF %>%
  mutate(GRM_mortality=ifelse(death==F & relapse==F & pod=="N", 0,
                              ifelse(relapse==T | pod =="Y" | (cod=="Disease Progression"|cod=="R
  mutate(GRM_mortality = ifelse(is.na(GRM_mortality),3,GRM_mortality)) %>%
  mutate(GRM_mortality = factor(GRM_mortality,levels=c(0,1,2,3),labels=c("Censored", "GRM", "Rela
  mutate(TRM_mortality=ifelse(death==F & relapse==F & pod=="N", 0,
                              ifelse(relapse==T | pod =="Y" | (cod=="Disease Progression"|cod=="R
  mutate(TRM_mortality = ifelse(is.na(TRM_mortality),1,TRM_mortality)) %>%
  mutate(TRM_mortality = factor(TRM_mortality,levels=c(0,1,2),labels=c("Censored","TRM","Relapse/

table(patients_urso_CIF2$GRM_mortality)
```

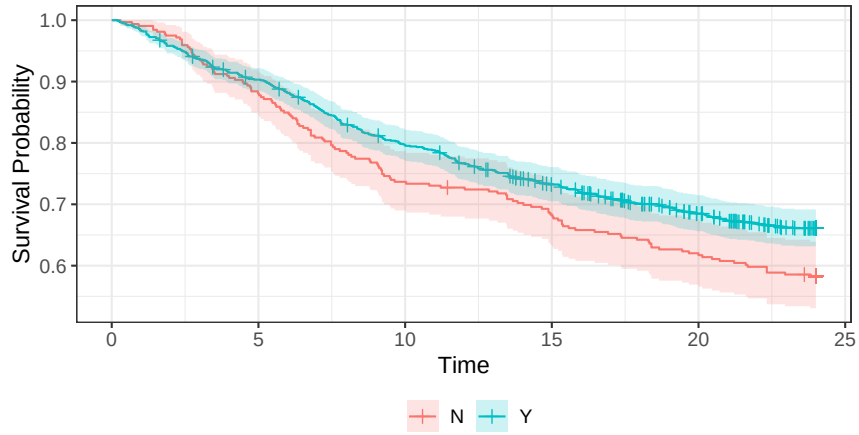
```
##
##    Censored         GRM Relapse/PoD       Other
##         624         191         386         100
```

```
patients_urso_CIF2$GRM_time <- patients_urso_CIF2$OS
patients_urso_CIF2$GRM_time[patients_urso_CIF2$relapse==T & patients_urso_CIF2$pod=="N"
patients_urso_CIF2$GRM_time[patients_urso_CIF2$relapse==F & patients_urso_CIF2$pod=="Y"
patients_urso_CIF2$GRM_time[patients_urso_CIF2$relapse==T & patients_urso_CIF2$pod=="Y"


patients_urso_CIF2$TRM_time <- patients_urso_CIF2$OS
patients_urso_CIF2$TRM_time[patients_urso_CIF2$relapse==T & patients_urso_CIF2$pod=="N"
patients_urso_CIF2$TRM_time[patients_urso_CIF2$relapse==F & patients_urso_CIF2$pod=="Y"
patients_urso_CIF2$TRM_time[patients_urso_CIF2$relapse==T & patients_urso_CIF2$pod=="Y"


patients_urso_CIF2 <- patients_urso_CIF2 %>%
  mutate(GRM_mortality_2yr = ifelse(GRM_time > 24,1,GRM_mortality),
         GRM_time_2yr = ifelse(GRM_time > 24,24,GRM_time),
         TRM_mortality_2yr = ifelse(TRM_time > 24,1,TRM_mortality),
         TRM_time_2yr = ifelse(TRM_time > 24,24,TRM_time),
         death_2yr = ifelse(OS > 24,FALSE,death),
         OS_2yr = ifelse(OS > 24,24,OS),
         GRM_mortality_2yr = factor(GRM_mortality_2yr,levels=1:4,labels=c("Censored","C
         TRM_mortality_2yr = factor(TRM_mortality_2yr,levels=1:3,labels=c("Censored",

patients_urso_CIF2 <- patients_urso_CIF2 %>% mutate(donor_new=ifelse(donor_match=="MMRD
```

8.2 Evaluate ursodiol exposure and overall survival

8.2.1 Univariable analysis

```
KM.OS <- survfit2(Surv(OS_2yr,as.numeric(death_2yr))~ursodiol2,data=patients_urso_CIF2)
KM.OS %>% ggsurvfit() +
  add_censor_mark() +
  add_confidence_interval() +
  add_risktable(times=c(0,6, 12, 18, 24))
```



N					
At Risk	319	271	231	204	184
Events	0	48	87	114	133
Y					
At Risk	982	860	743	646	551
Events	0	116	228	290	326

8.2.2 Multivariable analysis

```
coxfit <- survival::coxph(Surv(OS_2yr,as.numeric(death_2yr))~age+sex+donor_match+graft+intensity+
coxfit %>% tbl_regression(exponentiate=TRUE) %>% bold_p()
```

Characteristic	**HR**	**95% CI**	**p-value**
age	1.03	1.02, 1.04	___<0.001___
sex			
F	—	—	
M	0.95	0.79, 1.15	0.6
donor_match			
haplo	—	—	
haplo/MMUD	1.20	0.57, 2.51	0.6
MMRD	1.23	0.16, 9.28	0.8
MMUD	1.23	0.73, 2.07	0.4
MRD	0.74	0.45, 1.21	0.2
MUD	0.82	0.51, 1.32	0.4
graft			
BM	—	—	
CD34	0.89	0.60, 1.33	0.6
PBSC	1.15	0.80, 1.67	0.5
UCB	0.75	0.44, 1.28	0.3
intensity			
Ablative	—	—	
Nonablative	0.49	0.33, 0.73	___<0.001___
Reduced Intensity	1.00	0.74, 1.35	>0.9
ursodiol2			
N	—	—	
Y	0.69	0.55, 0.85	___<0.001___

8.3 Evaluation of cumulative incidences

8.3.1 Cumulative incidence of GVHD-related mortality

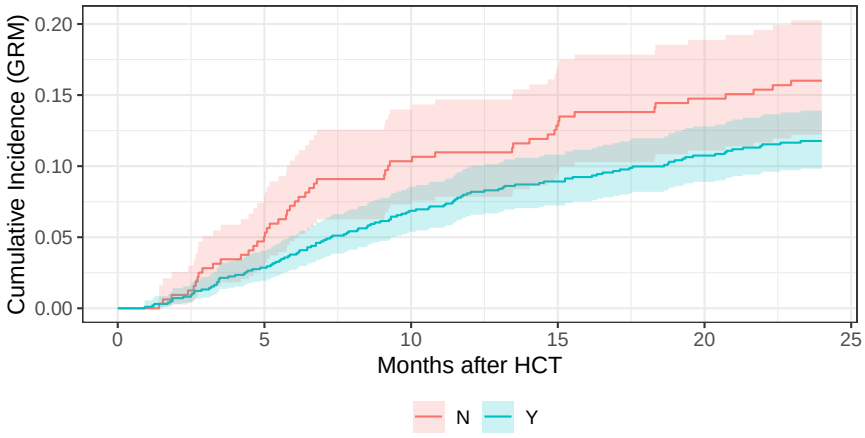
```
gray.test.GRM <- cuminc(Surv(GRM_time_2yr, GRM_mortality_2yr)~ursodiol2, data=patients_

gray.test.GRM %>% tbl_cuminc(times= c(6,12,18,24),outcome="GRM") %>%
  add_p() %>%
  add_n() %>%
  modify_caption("Outcome: GRM")
```

```
gray.test.GRM %>% ggcuminc(outcome="GRM") +
  labs(
    x = "Months after HCT",
    y = "Cumulative Incidence (GRM)"
  ) +
```

Table 8.1: Outcome: GRM

Characteristic	**N**	**Time 6**	**Time 12**	**Time 18**	**Time 24**	**
ursodiol2	1,301					
N		7.2% (4.7%, 10%)	11% (7.8%, 15%)	14% (10%, 18%)	16% (12%, 20%)	
Y		3.8% (2.7%, 5.1%)	8.1% (6.5%, 9.9%)	10% (8.2%, 12%)	12% (9.8%, 14%)	

```
  add_confidence_interval() +
  add_risktable(times=c(0,6,12,18,24))
```



N					
At Risk	319	243	201	177	160
Events	0	23	35	44	51
Y					
At Risk	982	757	613	541	456
Events	0	37	79	97	113

8.3.2 Cumulative incidence of Relapse/progression of disease

```
gray.test.GRM %>% tbl_cuminc(times= c(6,12,18,24),outcome="Relapse/PoD") %>%
  add_p() %>%
  add_n() %>%
  modify_caption("Outcome: Relapse/PoD")
```

Table 8.2: Outcome: Relapse/PoD

Characteristic	**N**	**Time 6**	**Time 12**	**Time 18**	**Time 24*
ursodiol2	1,301				
N		12% (8.9%, 16%)	18% (14%, 23%)	22% (17%, 26%)	23% (18%, 28
Y		15% (13%, 17%)	24% (21%, 26%)	26% (24%, 29%)	28% (25%, 3

Table 8.3: Outcome: Other

Characteristic	**N**	**Time 6**	**Time 12**	**Time 18**	**Tir
ursodiol2	1,301				
N		4.7% (2.7%, 7.4%)	7.2% (4.7%, 10%)	8.8% (6.0%, 12%)	11% (7.
Y		4.1% (3.0%, 5.4%)	5.0% (3.8%, 6.5%)	5.4% (4.1%, 7.0%)	5.6% (4.

8.3.3 Cumulative incidence of mortality non-related to GVHD or relapse/progression of disease

```
gray.test.GRM %>% tbl_cuminc(times= c(6,12,18,24),outcome="Other") %>%
  add_p() %>%
  add_n() %>%
  modify_caption("Outcome: Other")
```

8.3.4 Multivariable analysis of GVHD-related mortality

```
fgmodel.GRM <- crr(Surv(GRM_time_2yr,GRM_mortality_2yr)~age+sex+donor_match+graft+inter
fgmodel.GRM %>% tbl_regression(exponentiate=TRUE) %>% bold_p()
```

Characteristic	**HR**	**95% CI**	**p-value**
age	1.02	1.01, 1.04	___0.002___
sex			
F	—	—	
M	0.77	0.56, 1.05	0.10
donor_match			
haplo	—	—	
haplo/MMUD	1.78	0.57, 5.59	0.3
MMRD	0.00	0.00, 0.00	___<0.001___
MMUD	1.52	0.65, 3.58	0.3
MRD	0.58	0.26, 1.28	0.2
MUD	0.74	0.35, 1.59	0.4
graft			
BM	—	—	
CD34	1.28	0.59, 2.79	0.5
PBSC	1.64	0.85, 3.16	0.14
UCB	0.88	0.36, 2.19	0.8
intensity			
Ablative	—	—	
Nonablative	0.95	0.53, 1.71	0.9
Reduced Intensity	1.25	0.75, 2.09	0.4
ursodiol2			
N	—	—	
Y	0.66	0.46, 0.94	___0.022___