Hassoun et al_ctDNA in Testicular Cancer Clinical Analysis

library(swimplot) library(coxphf) library(grid) library(gtable) library(readr) library(mosaic) library(dplyr) library(survival) library(survminer) library(ggplot2) library(scales) library(coxphf) library(ggthemes) library(tidyverse) library(gtsummary) library(flextable) library(parameters) library(car) library(ComplexHeatmap) library(tidyverse) library(readxl) library(survival) library(janitor) library(rms) library(DT)

#ctDNA Detection rate by Stage and Window

Hide

```
#Pre-Orchiectomy
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$preSx.ctDNA.anytime!="",]
circ_data$preSx.ctDNA.anytime <- factor(circ_data$preSx.ctDNA.anytime, levels=c("NEGATIVE","POSITIVE"))
circ_data$Stage <- factor(circ_data$Stage, levels=c("I","II","III"))
circ_data <- subset(circ_data, preSx.ctDNA.anytime %in% c("NEGATIVE", "POSITIVE"))
positive_counts_by_stage <- aggregate(circ_data$preSx.ctDNA.anytime == "POSITIVE", by=list(circ_data$Stage), FUN=
sum)
total_counts_by_stage <- aggregate(circ_data$preSx.ctDNA.anytime, by=list(circ_data$Stage), FUN=length)
combined_data <- data.frame(
  Stage = total_counts_by_stage$Group.1,
  Total_Count = total_counts_by_stage$x,
  Positive_Count = positive_counts_by_stage$x,
  Rate = (positive_counts_by_stage$x / total_counts_by_stage$x) * 100  # Convert to percentage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$preSx.ctDNA.anytime == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Convert to percentage
overall_row <- data.frame(
  Stage = "Overall",
  Total_Count = overall_total_count,
  Positive_Count = overall_positive_count,
  Rate = sprintf("%.2f%%", overall_positivity_rate)
)
combined_data <- rbind(combined_data, overall_row)
print(combined_data)
```

Stage	Total_Count	Positive_Count	Rate
<fctr>	<int>	<int>	<chr>
I	12	11	91.67%
II	1	1	100.00%
III	2	2	100.00%
Overall	15	14	93.33%

4 rows

Hide

```
#MRD Window
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$ctDNA.MRD.Window!="",]
circ_data$ctDNA.MRD.Window <- factor(circ_data$ctDNA.MRD.Window, levels=c("NEGATIVE","POSITIVE"))
circ_data$Stage <- factor(circ_data$Stage, levels=c("I","II","III"))
circ_data <- subset(circ_data, ctDNA.MRD.Window %in% c("NEGATIVE", "POSITIVE"))
positive_counts_by_stage <- aggregate(circ_data$ctDNA.MRD.Window == "POSITIVE", by=list(circ_data$Stage), FUN=sum)
total_counts_by_stage <- aggregate(circ_data$ctDNA.MRD.Window, by=list(circ_data$Stage), FUN=length)
combined_data <- data.frame(
  Stage = total_counts_by_stage$Group.1,
  Total_Count = total_counts_by_stage$x,
  Positive_Count = positive_counts_by_stage$x,
  Rate = (positive_counts_by_stage$x / total_counts_by_stage$x) * 100  # Convert to percentage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$ctDNA.MRD.Window == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Convert to percentage
overall_row <- data.frame(
  Stage = "Overall",
  Total_Count = overall_total_count,
  Positive_Count = overall_positive_count,
  Rate = sprintf("%.2f%%", overall_positivity_rate)
)
combined_data <- rbind(combined_data, overall_row)
print(combined_data)
```

Stage	Total_Count	Positive_Count	Rate
<fctr>	<int>	<int>	<chr>
I	20	3	15.00%
II	9	7	77.78%
III	13	11	84.62%
Overall	42	21	50.00%

4 rows

Hide

```
#Surveillance Window
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$ctDNA.surveillance!="",]
circ_data$ctDNA.MRD.Window <- factor(circ_data$ctDNA.surveillance, levels=c("NEGATIVE","POSITIVE"))
circ_data$Stage <- factor(circ_data$Stage, levels=c("I","II","III"))
circ_data <- subset(circ_data, ctDNA.surveillance %in% c("NEGATIVE", "POSITIVE"))
positive_counts_by_stage <- aggregate(circ_data$ctDNA.surveillance == "POSITIVE", by=list(circ_data$Stage), FUN=sum)
total_counts_by_stage <- aggregate(circ_data$ctDNA.surveillance, by=list(circ_data$Stage), FUN=length)
combined_data <- data.frame(
  Stage = total_counts_by_stage$Group.1,
  Total_Count = total_counts_by_stage$x,
  Positive_Count = positive_counts_by_stage$x,
  Rate = (positive_counts_by_stage$x / total_counts_by_stage$x) * 100  # Convert to percentage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$ctDNA.surveillance == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Convert to percentage
overall_row <- data.frame(
  Stage = "Overall",
  Total_Count = overall_total_count,
  Positive_Count = overall_positive_count,
  Rate = sprintf("%.2f%%", overall_positivity_rate)
)
combined_data <- rbind(combined_data, overall_row)
print(combined_data)
```

Stage	Total_Count	Positive_Count	Rate
<fctr>	<int>	<int>	<chr>
I	27	4	14.81%
II	12	4	33.33%
III	12	5	41.67%
Overall	51	13	25.49%

4 rows

Hide

```
#Anytime post-surgery
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$postSx.ctDNA.anytime!="",]
circ_data$postSx.ctDNA.anytime <- factor(circ_data$postSx.ctDNA.anytime, levels=c("NEGATIVE","POSITIVE"))
circ_data$Stage <- factor(circ_data$Stage, levels=c("I","II","III"))
circ_data <- subset(circ_data, postSx.ctDNA.anytime %in% c("NEGATIVE", "POSITIVE"))
positive_counts_by_stage <- aggregate(circ_data$postSx.ctDNA.anytime == "POSITIVE", by=list(circ_data$Stage), FUN
=sum)
total_counts_by_stage <- aggregate(circ_data$postSx.ctDNA.anytime, by=list(circ_data$Stage), FUN=length)
combined_data <- data.frame(
  Stage = total_counts_by_stage$Group.1,
  Total_Count = total_counts_by_stage$x,
  Positive_Count = positive_counts_by_stage$x,
  Rate = (positive_counts_by_stage$x / total_counts_by_stage$x) * 100  # Convert to percentage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$postSx.ctDNA.anytime == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Convert to percentage
overall_row <- data.frame(
  Stage = "Overall",
  Total_Count = overall_total_count,
  Positive_Count = overall_positive_count,
  Rate = sprintf("%.2f%%", overall_positivity_rate)
)
combined_data <- rbind(combined_data, overall_row)
print(combined_data)
```

Stage	Total_Count	Positive_Count	Rate
<fctr>	<int>	<int>	<chr>
I	29	5	17.24%
II	17	12	70.59%
III	26	21	80.77%
Overall	72	38	52.78%

4 rows

#Demographics Table

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]

circ_data_subset <- circ_data %>%
  select(
    Age,
    pT,
    Stage,
    Histology,
    Treatment,
    RPLND.Pathology,
    preSx.markers.anytime,
    Markers.postSx.anytime,
    PFS.Event,
    OS.Event,
    OS.months) %>%
  mutate(
    Age = as.numeric(Age),
    pT = factor(pT, levels = c("0","1", "2", "3", "X"), labels = c("pT0","pT1", "pT2", "pT3","pTX")),
    Stage = factor(Stage, levels = c("I", "II", "III")),
    Histology = factor(Histology, levels = c("Non seminoma", "Seminoma")),
    Treatment = factor(Treatment, levels = c("Surveillance", "Chemotherapy", "RPLND", "RPLND, Chemotherapy")),
    RPLND.Pathology = factor(RPLND.Pathology, levels = c("Negative", "Germ Cell/Teratoma")),
    preSx.markers.anytime = factor(preSx.markers.anytime, levels = c("NEGATIVE", "POSITIVE"), labels = c("Norma
l", "Elevated")),
    Markers.postSx.anytime = factor(Markers.postSx.anytime, levels = c("NEGATIVE", "POSITIVE"), labels = c("Norma
l", "Elevated")),
    PFS.Event = factor(PFS.Event, levels = c("FALSE", "TRUE"), labels = c("No Event", "Event")),
    OS.Event = factor(OS.Event, levels = c("FALSE", "TRUE"), labels = c("Alive", "Deceased")),
    OS.months = as.numeric(OS.months))
table1 <- circ_data_subset %>%
  tbl_summary(
    statistic = list(
      all_continuous() ~ "{median} ({p25} - {p75})",
      all_categorical() ~ "{n} ({p}%)"),
    label = list(
      Age ~ "Age",
      pT ~ "Pathological T Stage",
      Stage ~ "Overall Stage",
      Histology ~ "Histology",
      Treatment ~ "Post-surgery treatment",
      RPLND.Pathology ~ "RPLND Pathology",
      preSx.markers.anytime ~ "Serum Markers pre-op",
      Markers.postSx.anytime ~ "Serum Markers anytime post-op",
      PFS.Event ~ "Event During Follow-up",
      OS.Event ~ "Vital Status",
      OS.months ~ "Total Follow up (months)"),) %>%
  bold_labels()
table1
```

Characteristic	N = 74[1]
Age	34 (27 - 39)
Pathological T Stage	
pT0	1 (1.4%)
pT1	39 (53%)
pT2	28 (38%)
pT3	3 (4.1%)
pTX	3 (4.1%)
Overall Stage	
I	30 (41%)
II	17 (23%)
III	27 (36%)
Histology	
Non seminoma	51 (69%)
Seminoma	23 (31%)
Post-surgery treatment	
Surveillance	17 (23%)

[1] Median (IQR); n (%)

Characteristic	N = 74[1]
Chemotherapy	30 (41%)
RPLND	5 (6.8%)
RPLND, Chemotherapy	22 (30%)
RPLND Pathology	
Negative	10 (37%)
Germ Cell/Teratoma	17 (63%)
Unknown	47
Serum Markers pre-op	
Normal	19 (26%)
Elevated	55 (74%)
Serum Markers anytime post-op	
Normal	30 (41%)
Elevated	44 (59%)
Event During Follow-up	
No Event	39 (53%)
Event	35 (47%)
Vital Status	
Alive	69 (93%)
Deceased	5 (6.8%)
Total Follow up (months)	17 (12 - 25)

[1] Median (IQR); n (%)

```
fit1 <- as_flex_table(
  table1,
  include = everything(),
  return_calls = FALSE,
  strip_md_bold = TRUE)
```

Warning: The `strip_md_bold` argument of `as_flex_table()` is deprecated as of gtsummary 1.6.0.

```
fit1
```

Characteristic	N = 74[1]
Age	34 (27 - 39)
Pathological T Stage	
pT0	1 (1.4%)
pT1	39 (53%)
pT2	28 (38%)
pT3	3 (4.1%)
pTX	3 (4.1%)
Overall Stage	
I	30 (41%)
II	17 (23%)
III	27 (36%)
Histology	
Non seminoma	51 (69%)
Seminoma	23 (31%)
Post-surgery treatment	
Surveillance	17 (23%)
Chemotherapy	30 (41%)

[1] Median (IQR); n (%)

Characteristic	N = 74[1]
RPLND	5 (6.8%)
RPLND, Chemotherapy	22 (30%)
RPLND Pathology	
Negative	10 (37%)
Germ Cell/Teratoma	17 (63%)
Unknown	47
Serum Markers pre-op	
Normal	19 (26%)
Elevated	55 (74%)
Serum Markers anytime post-op	
Normal	30 (41%)
Elevated	44 (59%)
Event During Follow-up	
No Event	39 (53%)
Event	35 (47%)
Vital Status	
Alive	69 (93%)
Deceased	5 (6.8%)
Total Follow up (months)	17 (12 - 25)

[1]Median (IQR); n (%)

```
save_as_docx(fit1, path= "~/Downloads/table1.docx")
```

#Heatmap with Clinical & Genomics Factors

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data %>% arrange(Stage)
circ_datadf <- as.data.frame(circ_data)

ha <- HeatmapAnnotation(
  Stage = circ_data$Stage,
  Histology = circ_data$Histology,
  pT = circ_data$pT,
  Treatment = circ_data$Treatment,
  Platinum.Response = circ_data$Platinum.Response,
  PFS.Event = circ_data$PFS.Event,
  preSx.markers.anytime = circ_data$preSx.markers.anytime,
  Markers.MRD.Window = circ_data$Markers.MRD.Window,
  Markers.Surveillance = circ_data$Markers.Surveillance,
  Markers.post.ACT = circ_data$Markers.post.ACT,
  Markers.post.RPLND = circ_data$Markers.post.RPLND,
  Markers.postSx.anytime = circ_data$Markers.postSx.anytime,
  Markers.anytime = circ_data$Markers.anytime,
  preSx.ctDNA.anytime = circ_data$preSx.ctDNA.anytime,
  ctDNA.MRD.Window = circ_data$ctDNA.MRD.Window,
  ctDNA.surveillance = circ_data$ctDNA.surveillance,
  ctDNA.post.ACT = circ_data$ctDNA.post.ACT,
  ctDNA.post.RPLND = circ_data$ctDNA.post.RPLND,
  postSx.ctDNA.anytime = circ_data$postSx.ctDNA.anytime,
  ctDNA.anytime = circ_data$ctDNA.anytime,

  col = list(Stage = c("I" = "seagreen1", "II" = "orange", "III" = "purple"),
    Histology = c("Non seminoma" = "goldenrod" , "Seminoma" = "blue4"),
    pT = c("0" = "coral","1" = "coral", "2" ="darkgreen", "3" = "yellow3", "X" = "gray"),
    Treatment = c("Surveillance" = "lightblue", "Chemotherapy" = "orange2", "RPLND" ="khaki", "RPLND, Chemotherap
y" = "brown2"),
    Platinum.Response = c("SD/PD" = "red3", "PR/CR" ="blue"),
    PFS.Event = c("TRUE" = "red3", "FALSE" ="blue"),
    preSx.markers.anytime = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    Markers.MRD.Window = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    Markers.Surveillance = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    Markers.post.ACT = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    Markers.post.RPLND = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    Markers.postSx.anytime = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    Markers.anytime = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    preSx.ctDNA.anytime = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    ctDNA.MRD.Window = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    ctDNA.surveillance = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    ctDNA.post.ACT = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    ctDNA.post.RPLND = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    postSx.ctDNA.anytime = c("POSITIVE" = "red3", "NEGATIVE" ="blue"),
    ctDNA.anytime = c("POSITIVE" = "red3", "NEGATIVE" ="blue")
)
)
ht <- Heatmap(matrix(nrow = 0, ncol = length(circ_data$Stage)),show_row_names = FALSE,cluster_rows = F,cluster_co
lumns = FALSE, top_annotation = ha)
pdf("heatmap.pdf",width = 7, height = 7)
draw(ht, annotation_legend_side = "bottom")
dev.off()
```

```
null device
          1
```
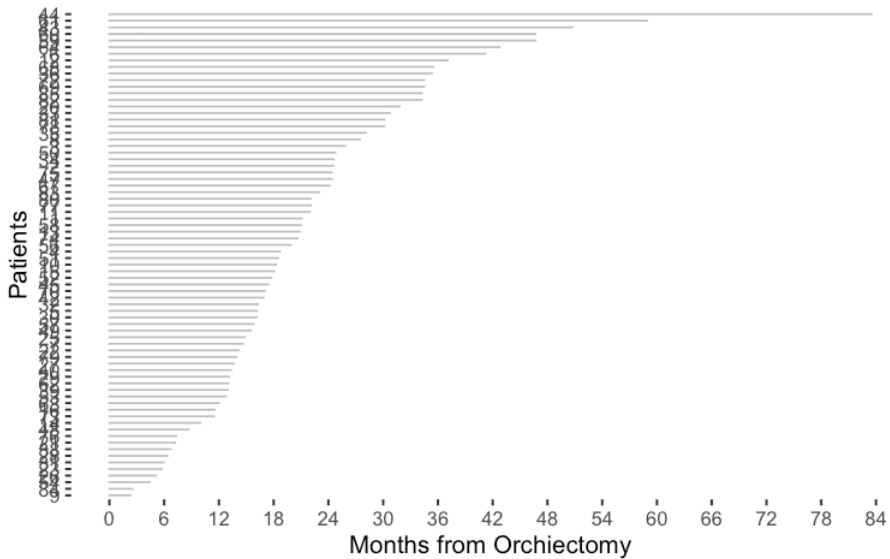
#Overview plot

Hide

```
library(swimplot)
library(ggplot2)
library(grid)
library(gtable)

#Overview plot
rm(list=ls())
setwd("~/Downloads")
clinstage<- read.csv("CLIA Testicular_OP.csv")
clinstage_df<- as.data.frame(clinstage)

oplot_stratify <-swimmer_plot(df=clinstage_df,
                              id='PatientName',
                              end='fu.diff.months',
                              #name_fill='Arm',
                              col="gray",
                              alpha=0.75,
                              width=.01,
                              base_size = 14,
                              )
oplot_stratify <- oplot_stratify + theme(panel.border = element_blank())
oplot_stratify <- oplot_stratify + scale_y_continuous(breaks = seq(0, 108, by = 6))
oplot_stratify <- oplot_stratify + labs(x ="Patients" , y="Months from Orchiectomy")
oplot_stratify
```
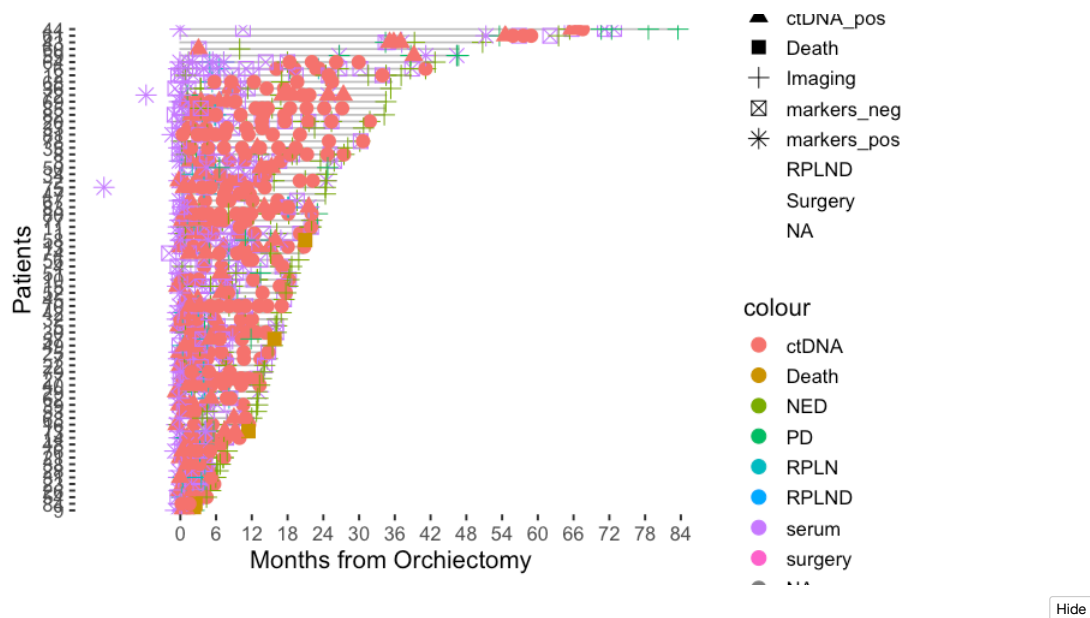

Hide

```
#plot events
oplot_ev3 <- oplot_stratify + swimmer_points(df_points=clinstage_df,
                                             id='PatientName',
                                             time='date.diff.months',
                                             name_shape ='Event_type',
                                             name_col = 'Event',
                                             size=3.5,fill='black',
                                             #col='darkgreen'
)
oplot_ev3
```
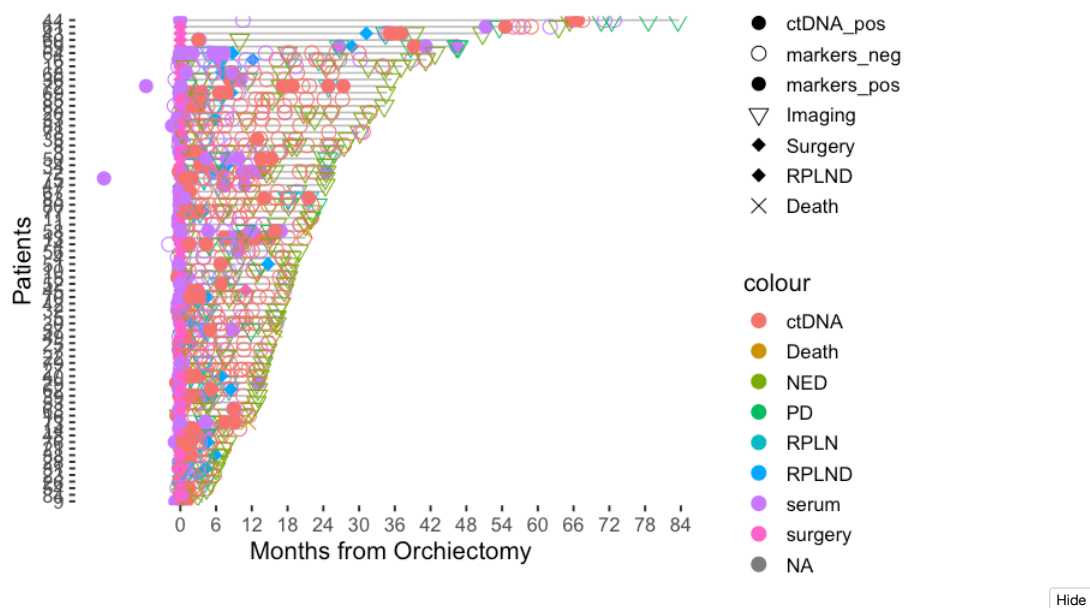
```
#Shape customization to Event_type

oplot_ev3.1 <- oplot_ev3 + ggplot2::scale_shape_manual(name="Event_type",values=c(1,16,1,16,6,18,18,4),breaks=c
('ctDNA_neg','ctDNA_pos','markers_neg','markers_pos','Imaging','Surgery','RPLND', 'Death'))

oplot_ev3.1
```
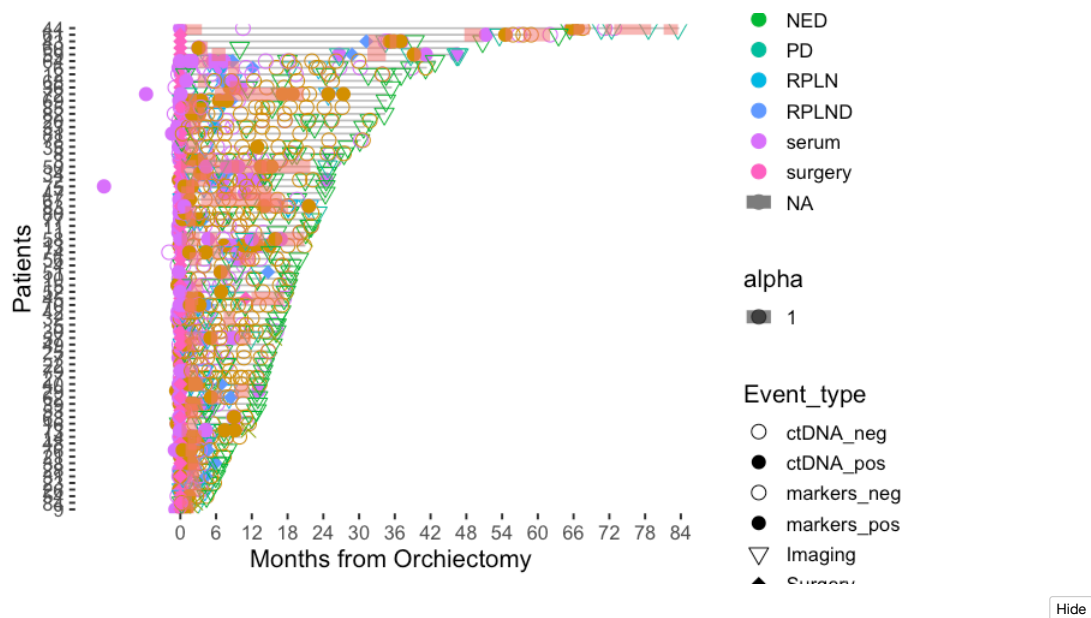
```
#plot treatment
oplot_ev4 <- oplot_ev3.1 + swimmer_lines(df_lines=clinstage_df,
                                    id='PatientName',
                                    start='Tx_start.months',
                                    end='Tx_end.months',
                                    name_col='Tx_type',
                                    size=3.5,
                                    name_alpha = 1.0)
oplot_ev4 <- oplot_ev4 + guides(linetype = guide_legend(override.aes = list(size = 5, color = "black")))
oplot_ev4
```

Hide

```
#colour customization
oplot_ev4.2 <- oplot_ev4 + ggplot2::scale_color_manual(name="Event",values=c( "lightblue", "black", "black", "gre
en", "red", "orange", "grey", "purple", "blue"))
oplot_ev4.2
```
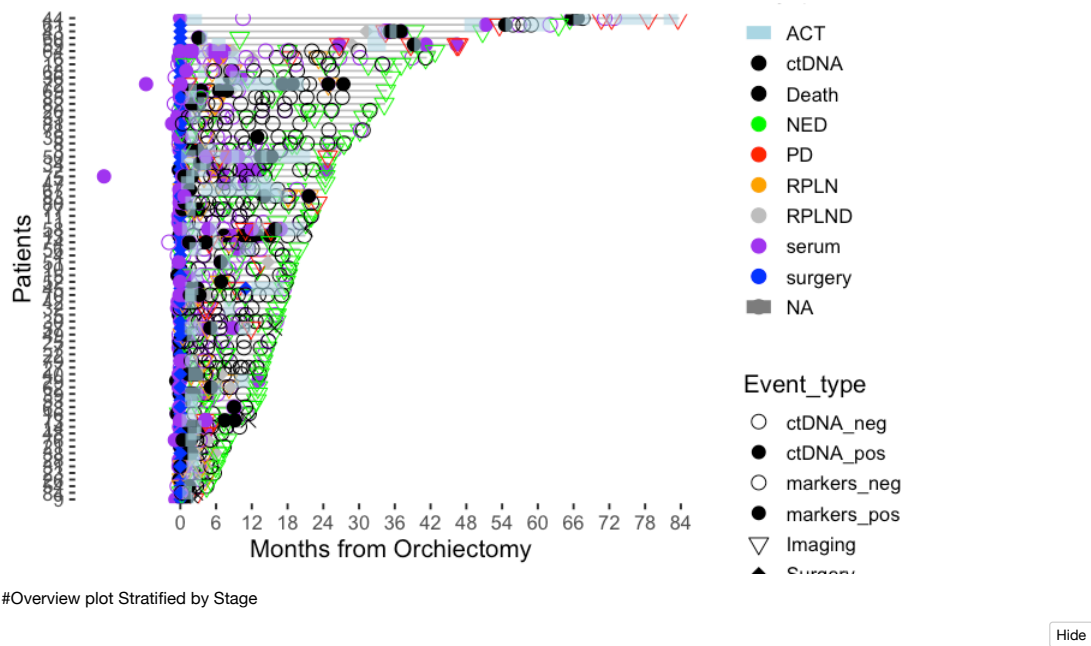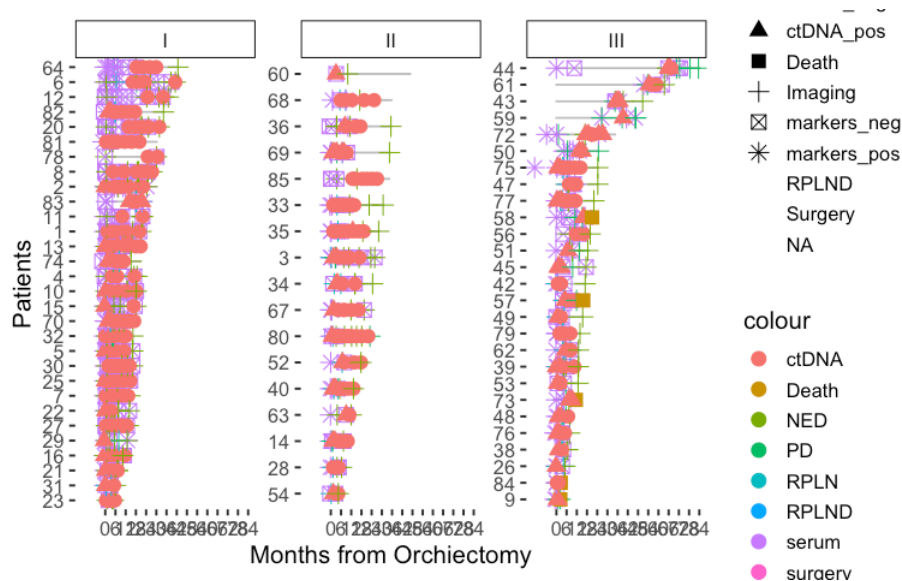

#Overview plot Stratified by Stage

Hide

```
library(swimplot)
library(ggplot2)
library(grid)
library(gtable)

#Overview plot
rm(list=ls())
setwd("~/Downloads")
clinstage<- read.csv("CLIA Testicular_OP.csv")
clinstage_df<- as.data.frame(clinstage)

oplot_stratify <-swimmer_plot(df=clinstage_df,
                              id='PatientName',
                              end='fu.diff.months',
                              #name_fill='Arm',
                              col="gray",
                              alpha=0.75,
                              width=.01,
                              base_size = 14,
                              stratify= c('Stage'))
oplot_stratify <- oplot_stratify + theme(panel.border = element_blank())
oplot_stratify <- oplot_stratify + scale_y_continuous(breaks = seq(0, 108, by = 6))
oplot_stratify <- oplot_stratify + labs(x ="Patients" , y="Months from Orchiectomy")
oplot_stratify
```


<div style="text-align:right">Hide</div>

```
#plot events
oplot_ev3 <- oplot_stratify + swimmer_points(df_points=clinstage_df,
                                             id='PatientName',
                                             time='date.diff.months',
                                             name_shape ='Event_type',
                                             name_col = 'Event',
                                             size=3.5,fill='black',
                                             #col='darkgreen'
)
oplot_ev3
```
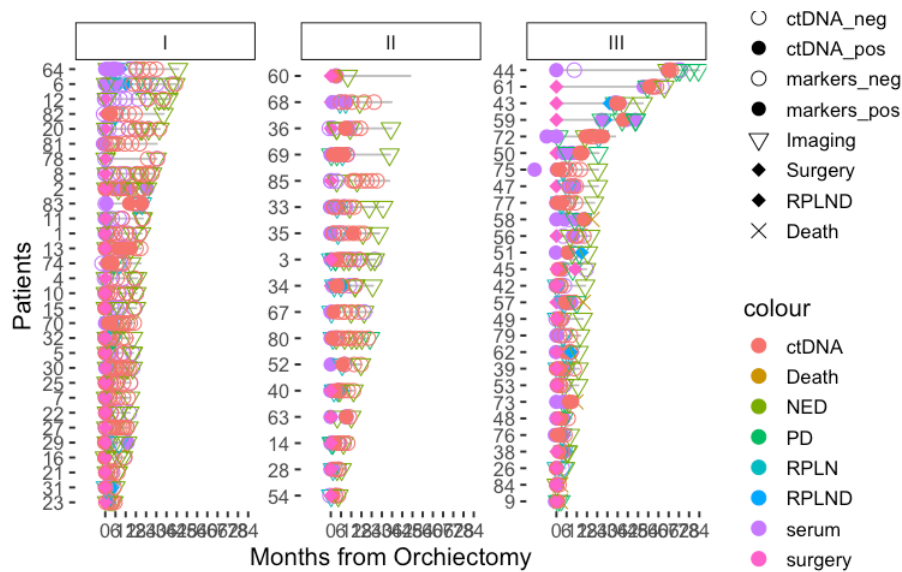
```
#Shape customization to Event_type

oplot_ev3.1 <- oplot_ev3 + ggplot2::scale_shape_manual(name="Event_type",values=c(1,16,1,16,6,18,18,4),breaks=c
('ctDNA_neg','ctDNA_pos','markers_neg','markers_pos','Imaging','Surgery','RPLND', 'Death'))

oplot_ev3.1
```

Hide

Hide

```
#plot treatment
oplot_ev4 <- oplot_ev3.1 + swimmer_lines(df_lines=clinstage_df,
                                        id='PatientName',
                                        start='Tx_start.months',
                                        end='Tx_end.months',
                                        name_col='Tx_type',
                                        size=3.5,
                                        name_alpha = 1.0)
oplot_ev4 <- oplot_ev4 + guides(linetype = guide_legend(override.aes = list(size = 5, color = "black")))
oplot_ev4
```
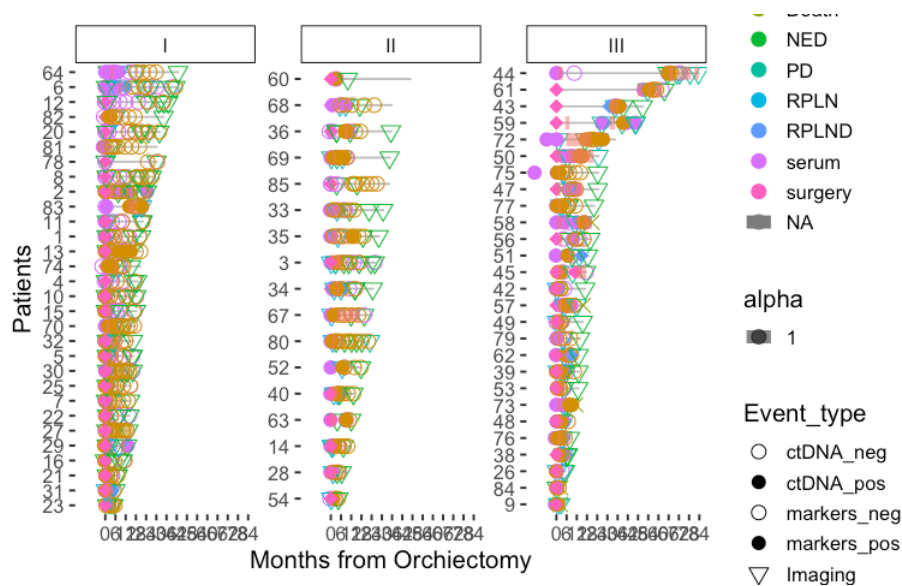
```
#colour customization
oplot_ev4.2 <- oplot_ev4 + ggplot2::scale_color_manual(name="Event",values=c( "lightblue", "black", "black", "gre
en", "red", "orange", "grey", "purple", "blue"))
oplot_ev4.2
```


#Sankey Plots for all pts with post-orchiectomy timepoint available

```
##To run this commands, please visit: https://sankeymatic.com/build/
#No pre-orch [20] MRD positive #E67272
#No pre-orch [12] MRD negative #87EA86
#No pre-orch [27] no MRD timepoint #808080
#Pre-orch Positive [1] MRD positive #E67272
#Pre-orch Positive [8] MRD negative #87EA86
#Pre-orch Positive [5] no MRD timepoint #808080
#Pre-orch Negative [1] MRD negative #87EA86

#MRD positive [7] Surveillance positive #E67272
#MRD positive [7] Surveillance negative #87EA86
#MRD positive [7] no Surveillance timepoint #80808
#MRD negative [1] Surveillance positive #E67272
#MRD negative [18] Surveillance negative #87EA86
#MRD negative [2] no Surveillance timepoint #80808
#no MRD timepoint [5] Surveillance positive #E67272
#no MRD timepoint [13] Surveillance negative #87EA86
#no MRD timepoint [14] no Surveillance timepoint #808080

#Surveillance positive [13] Event #E67272
#Surveillance positive [0] No Event #87EA86
#Surveillance negative [5] Event #E67272
#Surveillance negative [33] No Event #87EA86
#no Surveillance timepoint [17] Event #E67272
#no Surveillance timepoint [6] No Event #87EA86
```

#EFS by ctDNA at the MRD Window

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$ctDNA.MRD.Window!="",]
circ_datadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~ctDNA.MRD.Window, data = circ_data)
```

```
Call: survfit(formula = Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event) ~
    ctDNA.MRD.Window, data = circ_data)

                          n events median 0.95LCL 0.95UCL
ctDNA.MRD.Window=NEGATIVE 21      3     NA      NA      NA
ctDNA.MRD.Window=POSITIVE 21     13   5.36    4.07      NA
```

Hide

```
event_summary <- circ_data %>%
  group_by(ctDNA.MRD.Window) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

| ctDNA.MRD.Window | Total | Events | Fraction | Percentage |
<chr>	<int>	<int>	<dbl>	<dbl>
NEGATIVE	21	3	0.1428571	14.28571
POSITIVE	21	13	0.6190476	61.90476

2 rows

Hide

```
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.MRD.Window, data = circ_data,conf.int=0.95,conf.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=6, palett
e=c("blue","red"), title="EFS – ctDNA MRD window", ylab= "Event-Free Survival", xlab="Months from Orchiectomy", l
egend.labs=c("ctDNA Negative", "ctDNA Positive"), legend.title="")
```

EFS - ctDNA MRD window
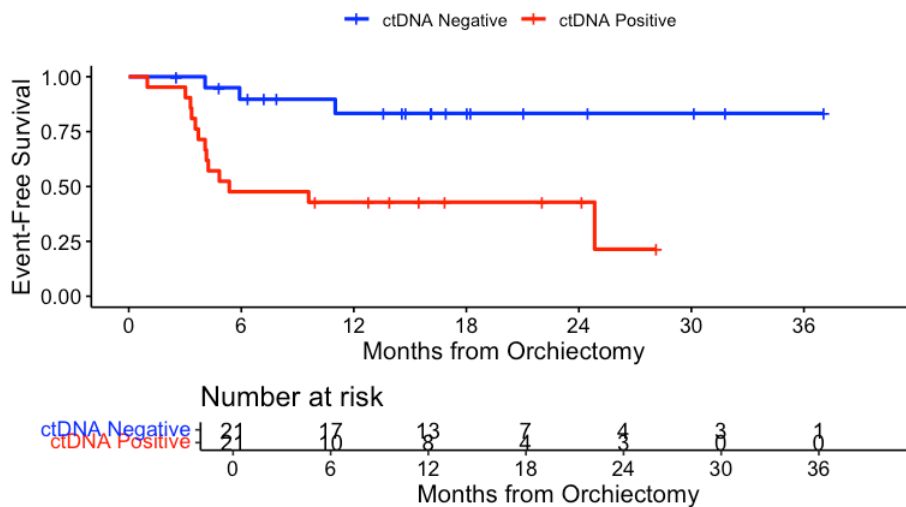

```
summary(KM_curve, times= c(12, 24))
```

```
Call: survfit(formula = surv_object ~ ctDNA.MRD.Window, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

                ctDNA.MRD.Window=NEGATIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
   12     13       3    0.833  0.0889        0.563        0.944
   24      4       0    0.833  0.0889        0.563        0.944

                ctDNA.MRD.Window=POSITIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
   12      8      12    0.429   0.108        0.219        0.623
   24      3       0    0.429   0.108        0.219        0.623
```

```
circ_data$ctDNA.MRD.Window <- factor(circ_data$ctDNA.MRD.Window, levels=c("NEGATIVE","POSITIVE"))
circ_data$Disease.Type <- factor(circ_data$Disease.Type, levels = c("Primary", "Metastatic"))
circ_data$Histology <- factor(circ_data$Histology, levels = c("Non seminoma", "Seminoma"), labels = c("Non semino
ma", "Seminoma"))
cox_fit <- coxph(surv_object ~ ctDNA.MRD.Window + Disease.Type + Histology, data=circ_data)
ggforest(cox_fit,data = circ_data)
```

Hazard ratio

ctDNA.MRD.Window	NEGATIVE *(N=21)*	reference			
	POSITIVE *(N=21)*	5.11 *(1.31 - 19.9)*			0.019 *
Disease.Type	Primary *(N=32)*	reference			
	Metastatic *(N=10)*	1.10 *(0.35 - 3.4)*			0.867
Histology	Non seminoma *(N=26)*	reference			
	Seminoma *(N=16)*	0.76 *(0.21 - 2.7)*			0.674

 # Events: 16; Global p-value (Log-Rank): 0.016419
AIC: 103.62; Concordance Index: 0.71

0.2   0.5   1   2   5   10   20

Hide

```
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ ctDNA.MRD.Window + Disease.Type +
    Histology, data = circ_data)

  n= 42, number of events= 16

                            coef exp(coef) se(coef)      z Pr(>|z|)
ctDNA.MRD.WindowPOSITIVE  1.63048   5.10634  0.69515  2.346    0.019 *
Disease.TypeMetastatic    0.09773   1.10267  0.58170  0.168    0.867
HistologySeminoma        -0.27236   0.76158  0.64651 -0.421    0.674
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                         exp(coef) exp(-coef) lower .95 upper .95
ctDNA.MRD.WindowPOSITIVE    5.1063     0.1958    1.3074    19.945
Disease.TypeMetastatic      1.1027     0.9069    0.3526     3.448
HistologySeminoma           0.7616     1.3131    0.2145     2.704

Concordance= 0.707  (se = 0.053 )
Likelihood ratio test= 10.27  on 3 df,   p=0.02
Wald test            = 7.8  on 3 df,   p=0.05
Score (logrank) test = 9.96  on 3 df,   p=0.02
```

#EFS by STMs at the MRD Window

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$ctDNA.MRD.Window!="",]
circ_data <- circ_data[circ_data$Markers.MRD.Window!="",]
circ_datadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~Markers.MRD.Window, data = circ_data)
```

```
Call: survfit(formula = Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event) ~
    Markers.MRD.Window, data = circ_data)

                             n events median 0.95LCL 0.95UCL
Markers.MRD.Window=NEGATIVE 16      3     NA      NA      NA
Markers.MRD.Window=POSITIVE 22     11   24.8    4.14      NA
```

Hide

```
event_summary <- circ_data %>%
  group_by(Markers.MRD.Window) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

| Markers.MRD.Window <chr>	Total <int>	Events <int>	Fraction <dbl>	Percentage <dbl>
NEGATIVE	16	3	0.1875	18.75
POSITIVE	22	11	0.5000	50.00
2 rows				

Hide

```
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
KM_curve <- survfit(surv_object ~ Markers.MRD.Window, data = circ_data,conf.int=0.95,conf.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=6, palett
e=c("blue","red"), title="EFS – STM at MRD window", ylab= "Event-Free Survival", xlab="Months from Orchiectomy",
legend.labs=c("Normal", "Elevated"), legend.title="")
```


EFS - STM at MRD window

Hide

```
summary(KM_curve, times= c(12))
```
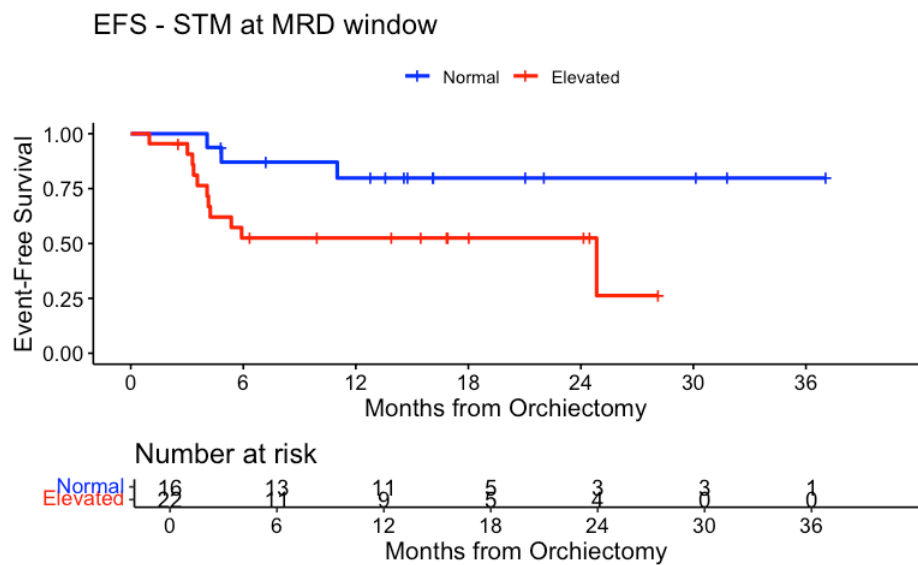
```
Call: survfit(formula = surv_object ~ Markers.MRD.Window, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

             Markers.MRD.Window=NEGATIVE
     time      n.risk     n.event     survival     std.err  lower 95% CI  upper 95% CI
   12.000      11.000       3.000        0.798       0.105         0.494         0.930

             Markers.MRD.Window=POSITIVE
     time      n.risk     n.event     survival     std.err  lower 95% CI  upper 95% CI
   12.000       9.000      10.000        0.525       0.109         0.298         0.710
```

Hide

```
circ_data$Markers.MRD.Window <- factor(circ_data$Markers.MRD.Window, levels=c("NEGATIVE","POSITIVE"))
circ_data$Disease.Type <- factor(circ_data$Disease.Type, levels = c("Primary", "Metastatic"))
circ_data$Histology <- factor(circ_data$Histology, levels = c("Non seminoma", "Seminoma"), labels = c("Non semino
ma", "Seminoma"))
cox_fit <- coxph(surv_object ~ Markers.MRD.Window + Disease.Type + Histology, data=circ_data)
ggforest(cox_fit,data = circ_data)
```

Hazard ratio

Markers.MRD.Window	NEGATIVE (N=16)	reference		
	POSITIVE (N=22)	2.97 (0.68 - 13.1)		0.149
Disease.Type	Primary (N=30)	reference		
	Metastatic (N=8)	1.11 (0.32 - 3.9)		0.868
Histology	Non seminoma (N=25)	reference		
	Seminoma (N=13)	0.68 (0.14 - 3.2)		0.625

Events: 14; Global p-value (Log-Rank): 0.15476
AIC: 92.54; Concordance Index: 0.67

0.2   0.5   1   2   5   10

Hide

```
summary(cox_fit)
```
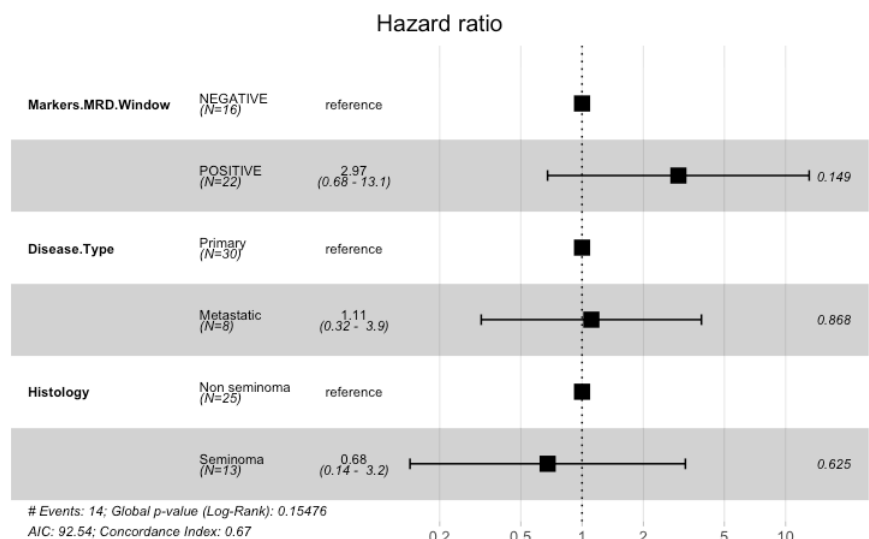
```
Call:
coxph(formula = surv_object ~ Markers.MRD.Window + Disease.Type +
    Histology, data = circ_data)

  n= 38, number of events= 14

                             coef exp(coef) se(coef)      z Pr(>|z|)
Markers.MRD.WindowPOSITIVE  1.0898    2.9738   0.7547  1.444    0.149
Disease.TypeMetastatic      0.1053    1.1110   0.6353  0.166    0.868
HistologySeminoma          -0.3885    0.6781   0.7945 -0.489    0.625

                           exp(coef) exp(-coef) lower .95 upper .95
Markers.MRD.WindowPOSITIVE    2.9738     0.3363    0.6775    13.054
Disease.TypeMetastatic        1.1110     0.9001    0.3198     3.859
HistologySeminoma             0.6781     1.4747    0.1429     3.218

Concordance= 0.669  (se = 0.061 )
Likelihood ratio test= 5.24  on 3 df,    p=0.2
Wald test            = 4.29  on 3 df,    p=0.2
Score (logrank) test = 4.91  on 3 df,    p=0.2
```

#EFS by ctDNA at the Surveillance Window

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$ctDNA.surveillance!="",]
circ_datadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~ctDNA.surveillance, data = circ_data)
```

```
Call: survfit(formula = Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event) ~
    ctDNA.surveillance, data = circ_data)

                          n events median 0.95LCL 0.95UCL
ctDNA.surveillance=NEGATIVE 38      5     NA      NA      NA
ctDNA.surveillance=POSITIVE 13     13   4.83    3.35      NA
```
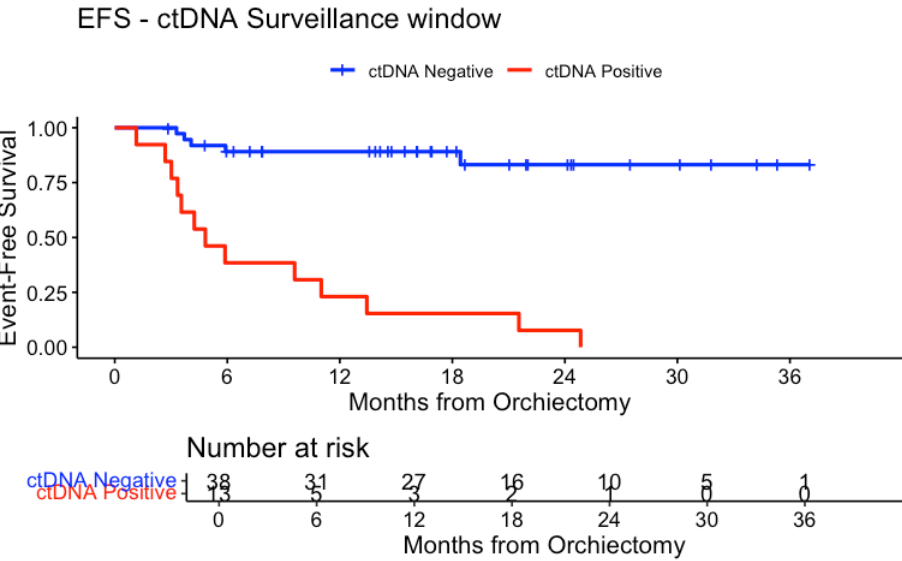
Hide

```
event_summary <- circ_data %>%
  group_by(ctDNA.surveillance) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

ctDNA.surveillance <chr>	Total <int>	Events <int>	Fraction <dbl>	Percentage <dbl>
NEGATIVE	38	5	0.1315789	13.15789
POSITIVE	13	13	1.0000000	100.00000

2 rows

Hide

```
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.surveillance, data = circ_data,conf.int=0.95,conf.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=6, palett
e=c("blue","red"), title="EFS - ctDNA Surveillance window", ylab= "Event-Free Survival", xlab="Months from Orchie
ctomy", legend.labs=c("ctDNA Negative", "ctDNA Positive"), legend.title="")
```


EFS - ctDNA Surveillance window

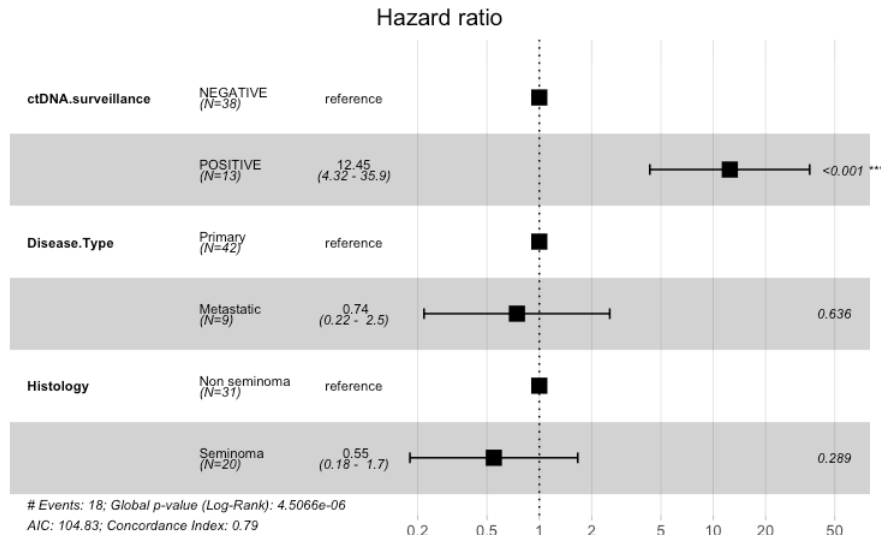Hide

```
summary(KM_curve, times= c(12))
```

```
Call: survfit(formula = surv_object ~ ctDNA.surveillance, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

            ctDNA.surveillance=NEGATIVE
     time     n.risk    n.event    survival    std.err lower 95% CI upper 95% CI
  12.0000    27.0000     4.0000      0.8911     0.0514       0.7352       0.9577

            ctDNA.surveillance=POSITIVE
     time     n.risk    n.event    survival    std.err lower 95% CI upper 95% CI
  12.0000     3.0000    10.0000      0.2308     0.1169       0.0558       0.4746
```

Hide

```
circ_data$ctDNA.surveillance <- factor(circ_data$ctDNA.surveillance, levels=c("NEGATIVE","POSITIVE"))
circ_data$Disease.Type <- factor(circ_data$Disease.Type, levels = c("Primary", "Metastatic"))
circ_data$Histology <- factor(circ_data$Histology, levels = c("Non seminoma", "Seminoma"), labels = c("Non semino
ma", "Seminoma"))
cox_fit <- coxph(surv_object ~ ctDNA.surveillance + Disease.Type + Histology, data=circ_data)
ggforest(cox_fit,data = circ_data)
```


Hide

```
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ ctDNA.surveillance + Disease.Type +
    Histology, data = circ_data)

  n= 51, number of events= 18

                                coef exp(coef) se(coef)      z Pr(>|z|)
ctDNA.surveillancePOSITIVE    2.5216   12.4485   0.5397  4.672 2.98e-06 ***
Disease.TypeMetastatic       -0.2967    0.7433   0.6270 -0.473    0.636
HistologySeminoma            -0.6017    0.5479   0.5670 -1.061    0.289
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                           exp(coef) exp(-coef) lower .95 upper .95
ctDNA.surveillancePOSITIVE   12.4485    0.08033    4.3222    35.854
Disease.TypeMetastatic        0.7433    1.34535    0.2175     2.540
HistologySeminoma             0.5479    1.82529    0.1803     1.665

Concordance= 0.793  (se = 0.069 )
Likelihood ratio test= 27.55  on 3 df,   p=5e-06
Wald test            = 23.95  on 3 df,   p=3e-05
Score (logrank) test = 38.6  on 3 df,   p=2e-08
```

#EFS by Serum Markers at the Surveillance Window

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$Markers.Surveillance!="",]
circ_datadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~Markers.Surveillance, data = circ_data)
```

```
Call: survfit(formula = Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event) ~
    Markers.Surveillance, data = circ_data)

                             n events median 0.95LCL 0.95UCL
Markers.Surveillance=NEGATIVE 48    15   65.3      NA      NA
Markers.Surveillance=POSITIVE 14    11   18.4    7.82      NA
```

<div align="right">Hide</div>

```
event_summary <- circ_data %>%
  group_by(Markers.Surveillance) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

Markers.Surveillance	Total	Events	Fraction	Percentage
<chr>	<int>	<int>	<dbl>	<dbl>
NEGATIVE	48	15	0.3125000	31.25000
POSITIVE	14	11	0.7857143	78.57143

2 rows

<div align="right">Hide</div>

```
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
KM_curve <- survfit(surv_object ~ Markers.Surveillance, data = circ_data,conf.int=0.95,conf.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=6, palett
e=c("blue","red"), title="EFS - Serum Markers Surveillance window", ylab= "Event-Free Survival", xlab="Months fro
m Orchiectomy", legend.labs=c("Normal", "Elevated"), legend.title="")
```


EFS - Serum Markers Surveillance window

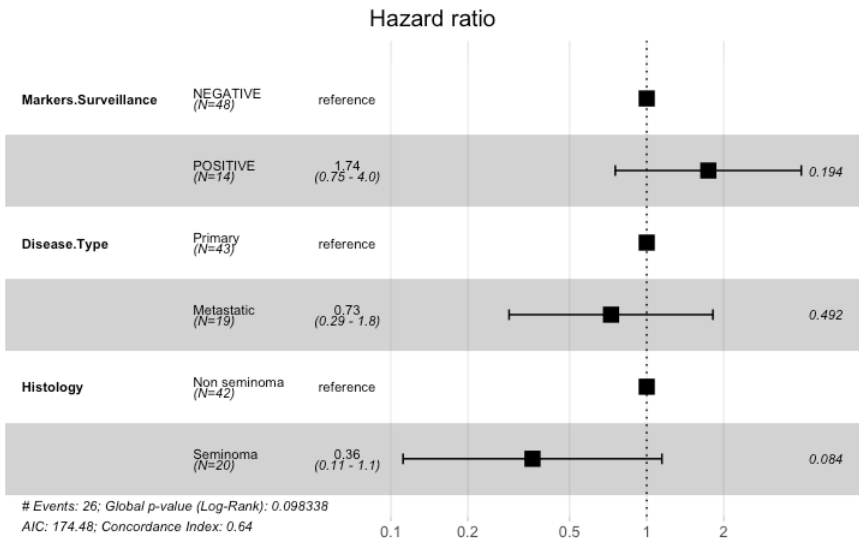```
summary(KM_curve, times= c(12))
```
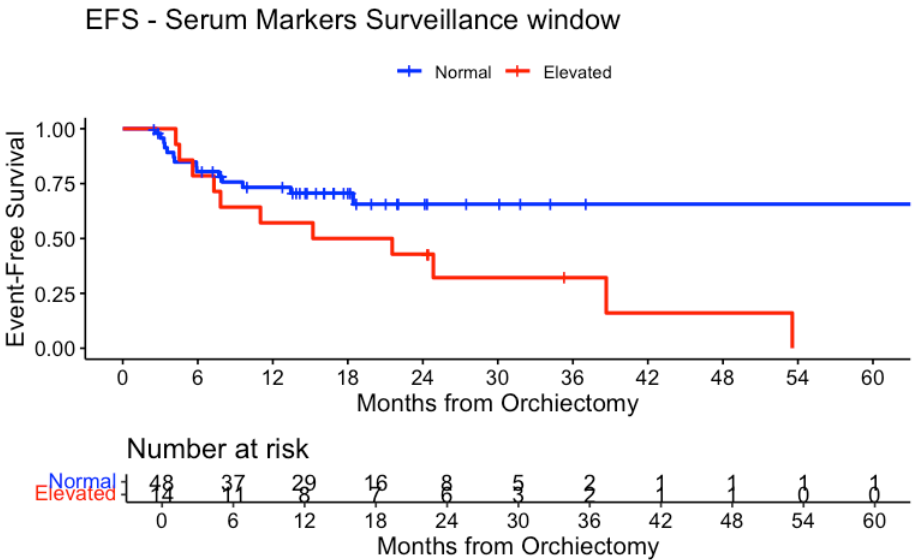
```
Call: survfit(formula = surv_object ~ Markers.Surveillance, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

            Markers.Surveillance=NEGATIVE
     time     n.risk    n.event    survival    std.err lower 95% CI upper 95% CI
  12.0000    29.0000    12.0000      0.7329     0.0663       0.5770       0.8389

            Markers.Surveillance=POSITIVE
     time     n.risk    n.event    survival    std.err lower 95% CI upper 95% CI
   12.000      8.000      6.000       0.571      0.132        0.284        0.780
```

```
circ_data$Markers.Surveillance <- factor(circ_data$Markers.Surveillance, levels=c("NEGATIVE","POSITIVE"))
circ_data$Disease.Type <- factor(circ_data$Disease.Type, levels = c("Primary", "Metastatic"))
circ_data$Histology <- factor(circ_data$Histology, levels = c("Non seminoma", "Seminoma"), labels = c("Non semino
ma", "Seminoma"))
cox_fit <- coxph(surv_object ~ Markers.Surveillance + Disease.Type + Histology, data=circ_data)
ggforest(cox_fit,data = circ_data)
```

```
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ Markers.Surveillance + Disease.Type +
    Histology, data = circ_data)

  n= 62, number of events= 26


                                coef exp(coef) se(coef)      z Pr(>|z|)
Markers.SurveillancePOSITIVE  0.5545    1.7410   0.4271  1.298   0.1942
Disease.TypeMetastatic       -0.3215    0.7251   0.4678 -0.687   0.4919
HistologySeminoma            -1.0279    0.3578   0.5944 -1.729   0.0838 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                             exp(coef) exp(-coef) lower .95 upper .95
Markers.SurveillancePOSITIVE    1.7410     0.5744    0.7538     4.021
Disease.TypeMetastatic          0.7251     1.3792    0.2899     1.814
HistologySeminoma               0.3578     2.7952    0.1116     1.147

Concordance= 0.638  (se = 0.05 )
Likelihood ratio test= 6.29  on 3 df,   p=0.1
Wald test            = 5.69  on 3 df,   p=0.1
Score (logrank) test = 6.19  on 3 df,   p=0.1
```

#EFS by ctDNA at post-RPLND - Stage II

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$RPLND==TRUE,]
circ_data <- circ_data[circ_data$Stage=="II",]
circ_data <- circ_data[circ_data$ctDNA.post.RPLND!="",]
circ_datadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~ctDNA.post.RPLND, data = circ_data)
```

```
Call: survfit(formula = Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event) ~
    ctDNA.post.RPLND, data = circ_data)

                          n events median 0.95LCL 0.95UCL
ctDNA.post.RPLND=NEGATIVE 4      2   7.69    5.91      NA
ctDNA.post.RPLND=POSITIVE 2      2   1.92    1.15      NA
```

Hide

```
event_summary <- circ_data %>%
  group_by(ctDNA.post.RPLND) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

ctDNA.post.RPLND <chr>	Total <int>	Events <int>	Fraction <dbl>	Percentage <dbl>
NEGATIVE	4	2	0.5	50
POSITIVE	2	2	1.0	100

2 rows

Hide

```
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.post.RPLND, data = circ_data,conf.int=0.95,conf.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = TRUE, conf.int = FALSE, risk.table = TRUE, break.time.by=3, palette
=c("blue","red"), title="EFS – ctDNA post-RPLND – Stage II", ylab= "Event–Free Survival", xlab="Months from Orchi
ectomy", legend.labs=c("ctDNA Negative", "ctDNA Positive"), legend.title="")
```

EFS - ctDNA post-RPLND - Stage II
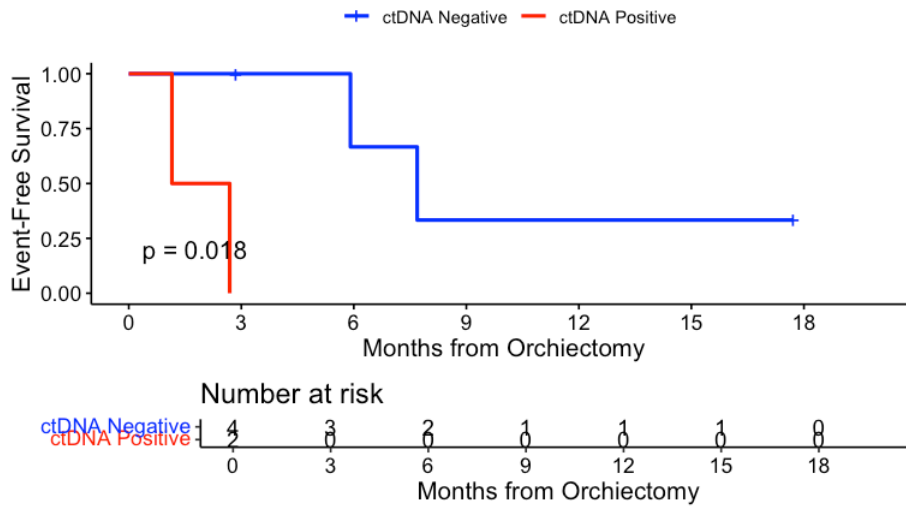
```
summary(KM_curve, times= c(0, 12))
```

```
Call: survfit(formula = surv_object ~ ctDNA.post.RPLND, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

              ctDNA.post.RPLND=NEGATIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0      4       0    1.000   0.000      1.00000        1.000
   12      1       2    0.333   0.272      0.00896        0.774

              ctDNA.post.RPLND=POSITIVE
      time     n.risk    n.event    survival     std.err lower 95% CI upper 95% CI
         0          2          0           1           0            1            1
```

```
circ_data$ctDNA.post.RPLND <- factor(circ_data$ctDNA.post.RPLND, levels=c("NEGATIVE","POSITIVE"))
cox_fit <- coxphf(surv_object ~ ctDNA.post.RPLND, data=circ_data)
summary(cox_fit)
```

```
coxphf(formula = surv_object ~ ctDNA.post.RPLND, data = circ_data)

Model fitted by Penalized ML
Confidence intervals and p-values by Profile Likelihood

                         coef se(coef) exp(coef) lower 0.95 upper 0.95    Chisq           p
ctDNA.post.RPLNDPOSITIVE 2.715398 1.929072  15.11062    1.165602   2114.922 4.34548 0.03710731

Likelihood ratio test=4.34548 on 1 df, p=0.03710731, n=6
Wald test = 1.981391 on 1 df, p = 0.159244

Covariance-Matrix:
                         ctDNA.post.RPLNDPOSITIVE
ctDNA.post.RPLNDPOSITIVE                 3.721317
```

#EFS by ctDNA at post-ACT - Stage II/III

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$ACT==TRUE,]
circ_data <- circ_data[circ_data$Stage!="I",]
circ_data <- circ_data[circ_data$ctDNA.surveillance!="",]
circ_dataadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~ctDNA.surveillance, data = circ_data)
```

```
Call: survfit(formula = Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event) ~
    ctDNA.surveillance, data = circ_data)

                            n events median 0.95LCL 0.95UCL
ctDNA.surveillance=NEGATIVE 13      3     NA      NA      NA
ctDNA.surveillance=POSITIVE  9      9   4.24    3.35      NA
```

```
event_summary <- circ_data %>%
  group_by(ctDNA.surveillance) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

ctDNA.surveillance	Total	Events	Fraction	Percentage
<chr>	<int>	<int>	<dbl>	<dbl>
NEGATIVE	13	3	0.2307692	23.07692
POSITIVE	9	9	1.0000000	100.00000

2 rows

```
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.surveillance, data = circ_data,conf.int=0.95,conf.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=3, palett
e=c("blue","red"), title="EFS - ctDNA post-ACT - Stage II/III", ylab= "Event-Free Survival", xlab="Months from Or
chiectomy", legend.labs=c("ctDNA Negative", "ctDNA Positive"), legend.title="")
```

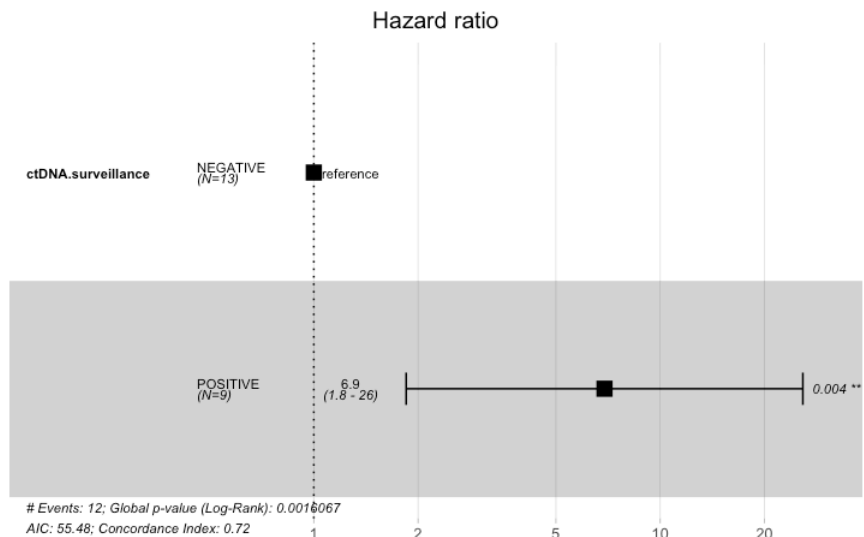```
summary(KM_curve, times= c(12, 24))
```
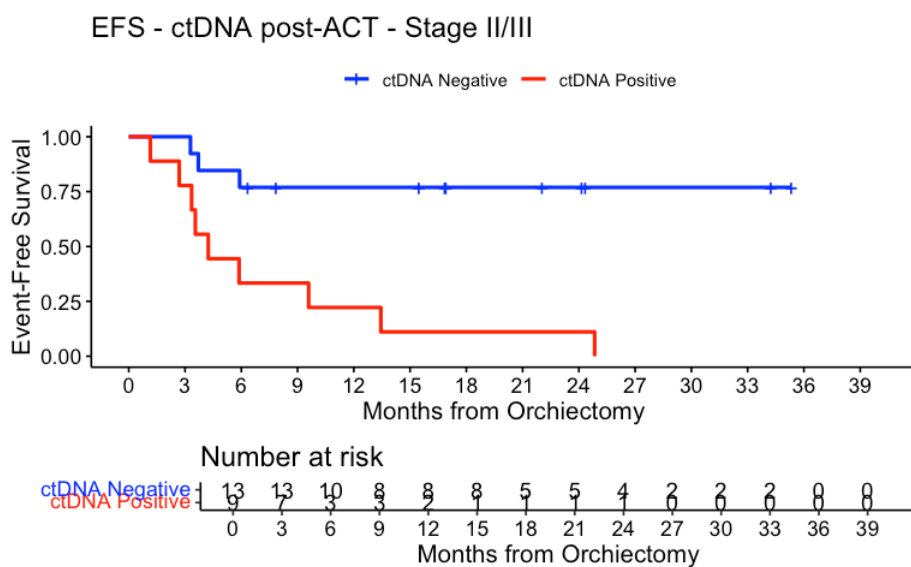
```
Call: survfit(formula = surv_object ~ ctDNA.surveillance, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

             ctDNA.surveillance=NEGATIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
   12      8       3    0.769   0.117        0.442        0.919
   24      4       0    0.769   0.117        0.442        0.919

             ctDNA.surveillance=POSITIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
   12      2       7    0.222   0.139      0.03371        0.513
   24      1       1    0.111   0.105      0.00613        0.388
```

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```
circ_data$ctDNA.surveillance <- factor(circ_data$ctDNA.surveillance, levels=c("NEGATIVE","POSITIVE"))
cox_fit <- coxph(surv_object ~ ctDNA.surveillance, data=circ_data)
ggforest(cox_fit,data = circ_data)
```


Hazard ratio

ctDNA.surveillance NEGATIVE (N=13) reference

POSITIVE (N=9) 6.9 (1.8 - 26) 0.004 **

Events: 12; Global p-value (Log-Rank): 0.0016067
AIC: 55.48; Concordance Index: 0.72

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```
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ ctDNA.surveillance, data = circ_data)

  n= 22, number of events= 12

                              coef exp(coef) se(coef)     z Pr(>|z|)
ctDNA.surveillancePOSITIVE 1.9323    6.9055   0.6727 2.873  0.00407 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                           exp(coef) exp(-coef) lower .95 upper .95
ctDNA.surveillancePOSITIVE     6.905     0.1448     1.848     25.81

Concordance= 0.716  (se = 0.067 )
Likelihood ratio test= 9.95  on 1 df,    p=0.002
Wald test            = 8.25  on 1 df,    p=0.004
Score (logrank) test = 10.97  on 1 df,    p=9e-04
```

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```
cox_fit_summary <- summary(cox_fit)

# Extract values for HR, 95% CI, and p-value
HR <- cox_fit_summary$coefficients[2]
lower_CI <- cox_fit_summary$conf.int[3]
upper_CI <- cox_fit_summary$conf.int[4]
p_value <- cox_fit_summary$coefficients[5]
label_text <- paste0("HR = ", round(HR, 2), " (", round(lower_CI, 2), "-", round(upper_CI, 2), "); p = ", round(p
_value, 3))
print(label_text)
```

```
[1] "HR = 6.91 (1.85-25.81); p = 0.004"
```

#EFS by STMs at post-ACT - Stage II/III

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```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("CLIA Testicular_GL.csv")
circ_data <- circ_data[circ_data$ctDNA.available==TRUE,]
circ_data <- circ_data[circ_data$ACT==TRUE,]
circ_data <- circ_data[circ_data$Stage!="I",]
circ_data <- circ_data[circ_data$Markers.Surveillance!="",]
circ_datadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~Markers.Surveillance, data = circ_data)
```

```
Call: survfit(formula = Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event) ~
    Markers.Surveillance, data = circ_data)

                              n events median 0.95LCL 0.95UCL
Markers.Surveillance=NEGATIVE 23     11   65.3    7.69      NA
Markers.Surveillance=POSITIVE 10      8   20.0    5.59      NA
```

Hide

```
event_summary <- circ_data %>%
  group_by(Markers.Surveillance) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```
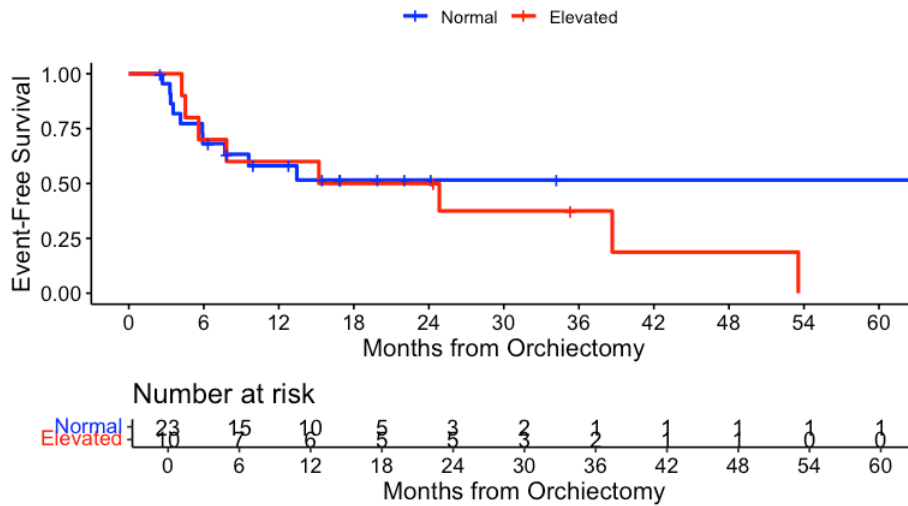
Markers.Surveillance	Total	Events	Fraction	Percentage
<chr>	<int>	<int>	<dbl>	<dbl>
NEGATIVE	23	11	0.4782609	47.82609
POSITIVE	10	8	0.8000000	80.00000

2 rows

Hide

```
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
KM_curve <- survfit(surv_object ~ Markers.Surveillance, data = circ_data,conf.int=0.95,conf.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=6, palett
e=c("blue","red"), title="EFS - STMs post-ACT - Stage II/III", ylab= "Event-Free Survival", xlab="Months from Orc
hiectomy", legend.labs=c("Normal", "Elevated"), legend.title="")
```

EFS - STMs post-ACT - Stage II/III

Legend: Normal (blue) / Elevated (red)

Y-axis: Event-Free Survival (1.00, 0.75, 0.50, 0.25, 0.00)
X-axis: Months from Orchiectomy (0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60)

Number at risk

	0	6	12	18	24	30	36	42	48	54	60
Normal	23	15	10	5	3	2	1	1	1	1	1
Elevated	10	7	6	5	5	3	2	1	0	0	

Months from Orchiectomy

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```
summary(KM_curve, times= c(12, 24))
```
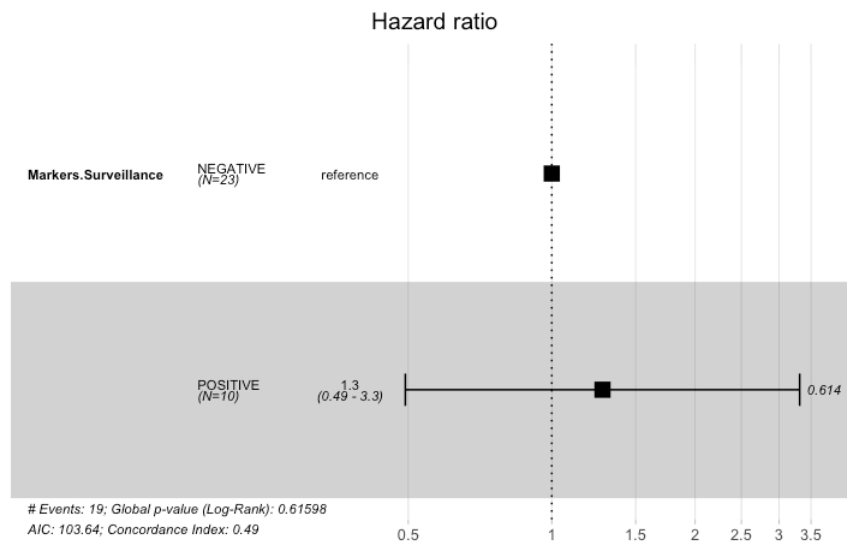
```
Call: survfit(formula = surv_object ~ Markers.Surveillance, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

          Markers.Surveillance=NEGATIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
   12     10       9    0.580   0.107        0.346        0.756
   24      3       1    0.516   0.113        0.281        0.708

          Markers.Surveillance=POSITIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
   12      6       4      0.6   0.155        0.253        0.827
   24      5       1      0.5   0.158        0.184        0.753
```

Hide

```
circ_data$Markers.Surveillance <- factor(circ_data$Markers.Surveillance, levels=c("NEGATIVE","POSITIVE"))
cox_fit <- coxph(surv_object ~ Markers.Surveillance, data=circ_data)
ggforest(cox_fit,data = circ_data)
```

Hazard ratio

Markers.Surveillance NEGATIVE (N=23) reference

POSITIVE (N=10) 1.3 (0.49 - 3.3) 0.614

Events: 19; Global p-value (Log-Rank): 0.61598
AIC: 103.64; Concordance Index: 0.49

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```
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ Markers.Surveillance, data = circ_data)

  n= 33, number of events= 19

                               coef exp(coef) se(coef)      z Pr(>|z|)
Markers.SurveillancePOSITIVE 0.2453    1.2780   0.4862 0.505    0.614

                             exp(coef) exp(-coef) lower .95 upper .95
Markers.SurveillancePOSITIVE     1.278     0.7825    0.4928     3.314

Concordance= 0.49  (se = 0.058 )
Likelihood ratio test= 0.25  on 1 df,   p=0.6
Wald test            = 0.25  on 1 df,   p=0.6
Score (logrank) test = 0.26  on 1 df,   p=0.6
```

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```
cox_fit_summary <- summary(cox_fit)

# Extract values for HR, 95% CI, and p-value
HR <- cox_fit_summary$coefficients[2]
lower_CI <- cox_fit_summary$conf.int[3]
upper_CI <- cox_fit_summary$conf.int[4]
p_value <- cox_fit_summary$coefficients[5]
label_text <- paste0("HR = ", round(HR, 2), " (", round(lower_CI, 2), "-", round(upper_CI, 2), "); p = ", round(p
_value, 3))
print(label_text)
```

```
[1] "HR = 1.28 (0.49-3.31); p = 0.614"
```