EORTC HNSCC ICI Honore et al_02032025

library(swimplot) 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(janitor) library(DT) library(pROC) library(rms)

#ctDNA Detection Rates by Window and Stages

Hide

```
#ctDNA at Baseline
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data$ctDNA.Base <- factor(circ_data$ctDNA.Base, levels=c("NEGATIVE","POSITIVE"))
circ_data <- subset(circ_data, ctDNA.Base %in% c("NEGATIVE", "POSITIVE"))
circ_data$Stage <- factor(circ_data$Stage, levels=c("0","I","II","III","IV"))
positive_counts_by_stage <- aggregate(circ_data$ctDNA.Base == "POSITIVE", by=list(circ_d
ata$Stage), FUN=sum)
total_counts_by_stage <- aggregate(circ_data$ctDNA.Base, by=list(circ_data$Stage), FUN=l
ength)
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$x) * 100  # Convert to perc
entage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$ctDNA.Base == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Conve
rt 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	1	1	100.00%
II	5	4	80.00%
III	3	3	100.00%
IV	19	17	89.47%
Overall	29	25	86.21%
5 rows			

Hide

```
#ctDNA post-treatment
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.postTx!="",]
circ_data$ctDNA.postTx <- factor(circ_data$ctDNA.postTx, levels=c("NEGATIVE","POSITIV
E"))
circ_data$Stage <- factor(circ_data$Stage, levels=c("0","I","II","III","IV"))
positive_counts_by_stage <- aggregate(circ_data$ctDNA.postTx == "POSITIVE", by=list(circ
_data$Stage), FUN=sum)
total_counts_by_stage <- aggregate(circ_data$ctDNA.postTx, by=list(circ_data$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$x) * 100  # Convert to perc
entage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$ctDNA.postTx == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Conve
rt 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	1	1	100.00%
II	5	1	20.00%
III	3	2	66.67%
IV	19	15	78.95%
Overall	29	20	68.97%

5 rows

#Overview plot

Hide

```
setwd("~/Downloads")
clinstage <- read.csv("EORTC ICI_OP.csv")
clinstage_df <- as.data.frame(clinstage)

# Creating the basic swimmer plot
oplot <- swimmer_plot(df=clinstage_df,
                      id='PatientName',
                      end='fu.diff.months',
                      fill='gray',
                      width=.01)

# Adding themes and scales
oplot <- oplot + theme(panel.border = element_blank())
oplot <- oplot + scale_y_continuous(breaks = seq(0, 48, by = 3))
oplot <- oplot + labs(x ="Patients", y="Months from Immunotherapy Start")

# Adding swimmer points
oplot_ev1 <- oplot + swimmer_points(df_points=clinstage_df,
                                    id='PatientName',
                                    time='date.diff.months',
                                    name_shape ='Event_type',
                                    name_col = 'Event',
                                    size=3.5,fill='black')
# Optionally uncomment and use col='darkgreen' if needed

# Adding shape manual scale
oplot_ev1.1 <- oplot_ev1 + ggplot2::scale_shape_manual(name="Event_type",
                                                       values=c(1,16,6,4),
                                                       breaks=c('ctDNA_neg','ctDNA_po
s','Imaging','Death'))

# Display the plot
oplot_ev1.1
```
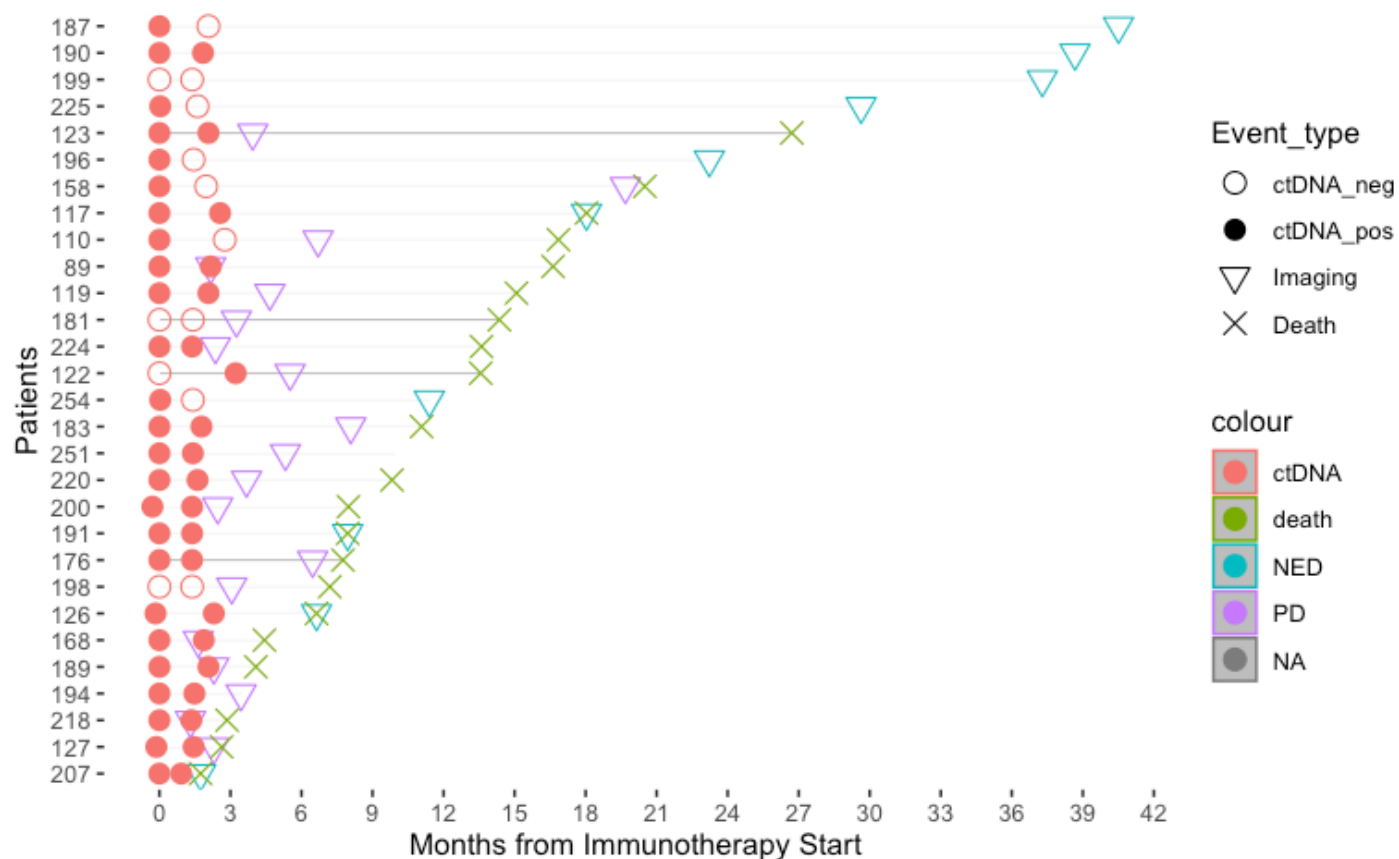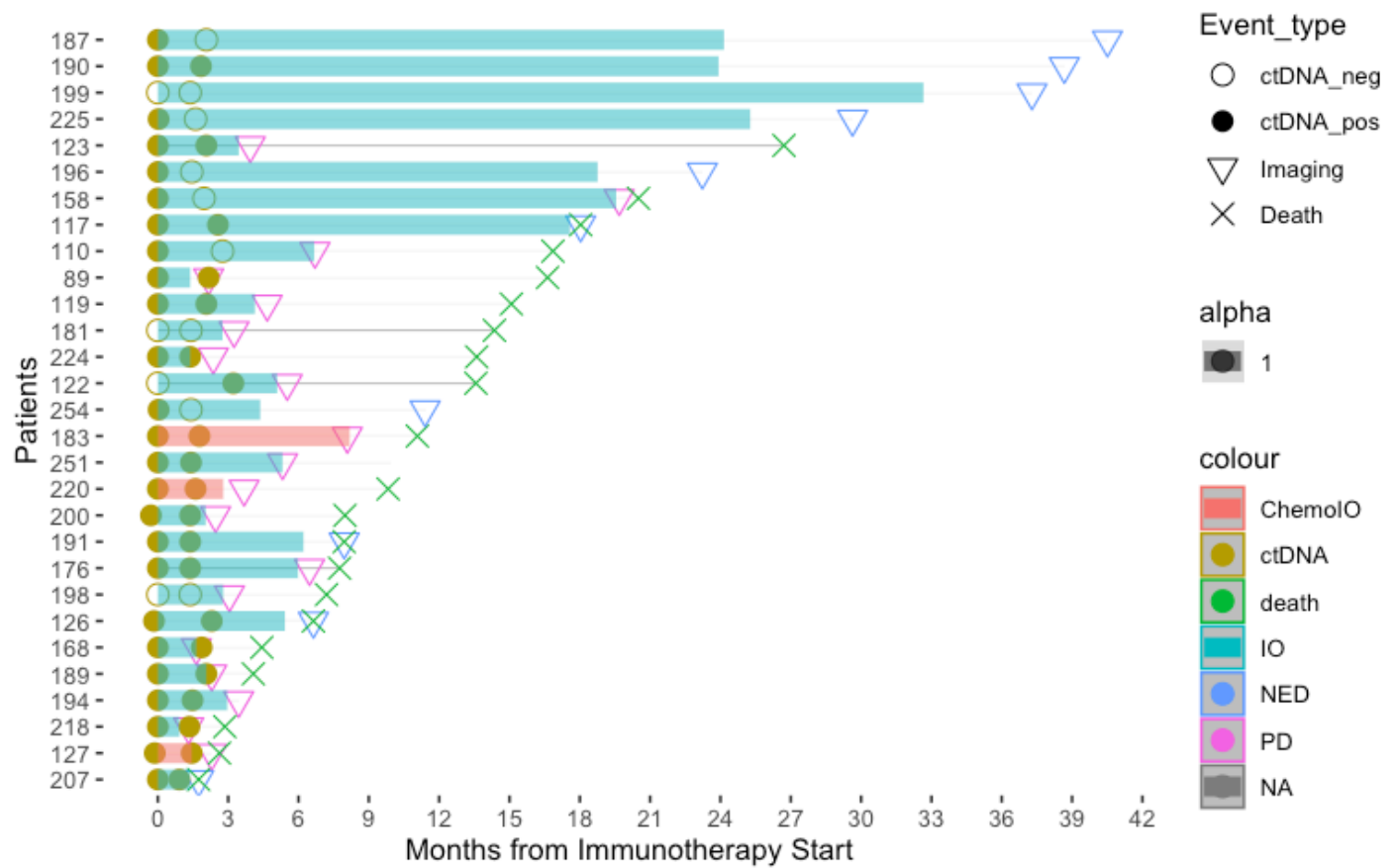
```
                                    oplot_ev2 <- oplot_ev1.1 + swimmer_lines(df_lines=cl
instage_df,
                                                                        id='Patient
Name',
                                                                        start='Tx_s
tart.months',
                                                                        end='Tx_en
d.months',
                                                                        name_col='T
x_type',
                                                                        size=3.5,
                                                                        name_alpha
= 1.0)
                                    oplot_ev2 <- oplot_ev2 + guides(linetype = guide_leg
end(override.aes = list(size = 5, color = "black")))
                                    oplot_ev2
```
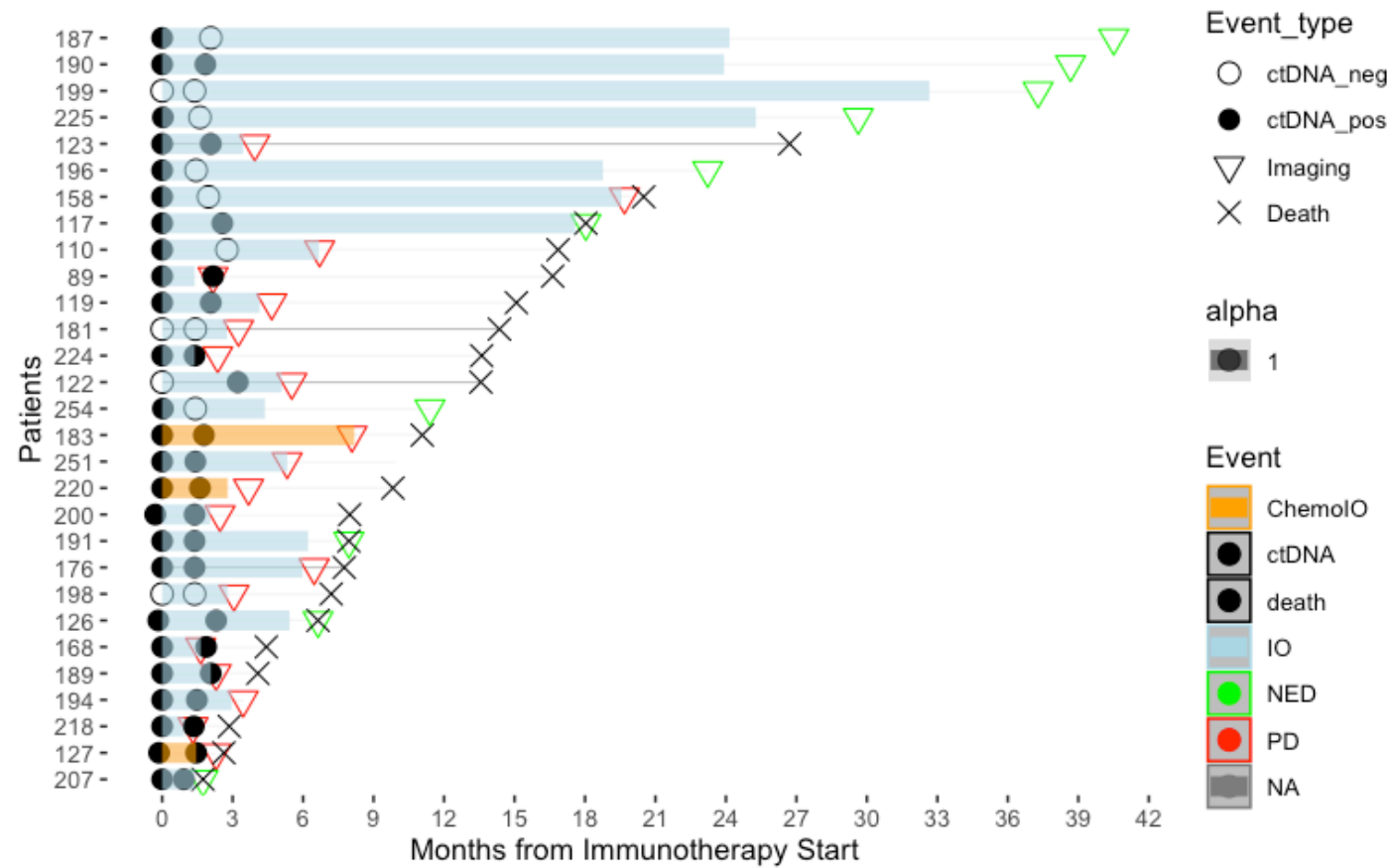
```
oplot_ev2.2 <- oplot_ev2 + ggplot2::scale_color_manual(name="Event",values=c( "orange",
"black", "black", "lightblue", "green", "red"))
oplot_ev2.2
```

#Overview plot - stratified by BOR

Hide

```
setwd("~/Downloads")
clinstage <- read.csv("EORTC ICI_OP.csv")
clinstage_df <- as.data.frame(clinstage)

# Creating the basic swimmer plot
oplot_stratify <-swimmer_plot(df=clinstage_df,
                              id='PatientName',
                              end='fu.diff.months',
                              col="gray",
                              alpha=0.75,
                              width=.01,
                              base_size = 14,
                              stratify= c('RECIST'))
oplot_stratify <- oplot_stratify + theme(panel.border = element_blank())
oplot_stratify <- oplot_stratify + scale_y_continuous(breaks = seq(0, 42, by = 3))
oplot_stratify <- oplot_stratify + labs(x ="Patients" , y="Months from Immunotherapy Sta
rt")

# Adding swimmer points
oplot_ev1 <- 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')
# Optionally uncomment and use col='darkgreen' if needed

# Adding shape manual scale
oplot_ev1.1 <- oplot_ev1 + ggplot2::scale_shape_manual(name="Event_type",
                                                  values=c(1,16,6,4),
                                                  breaks=c('ctDNA_neg','ctDNA_po
s','Imaging','Death'))

# Display the plot
oplot_ev1.1
```
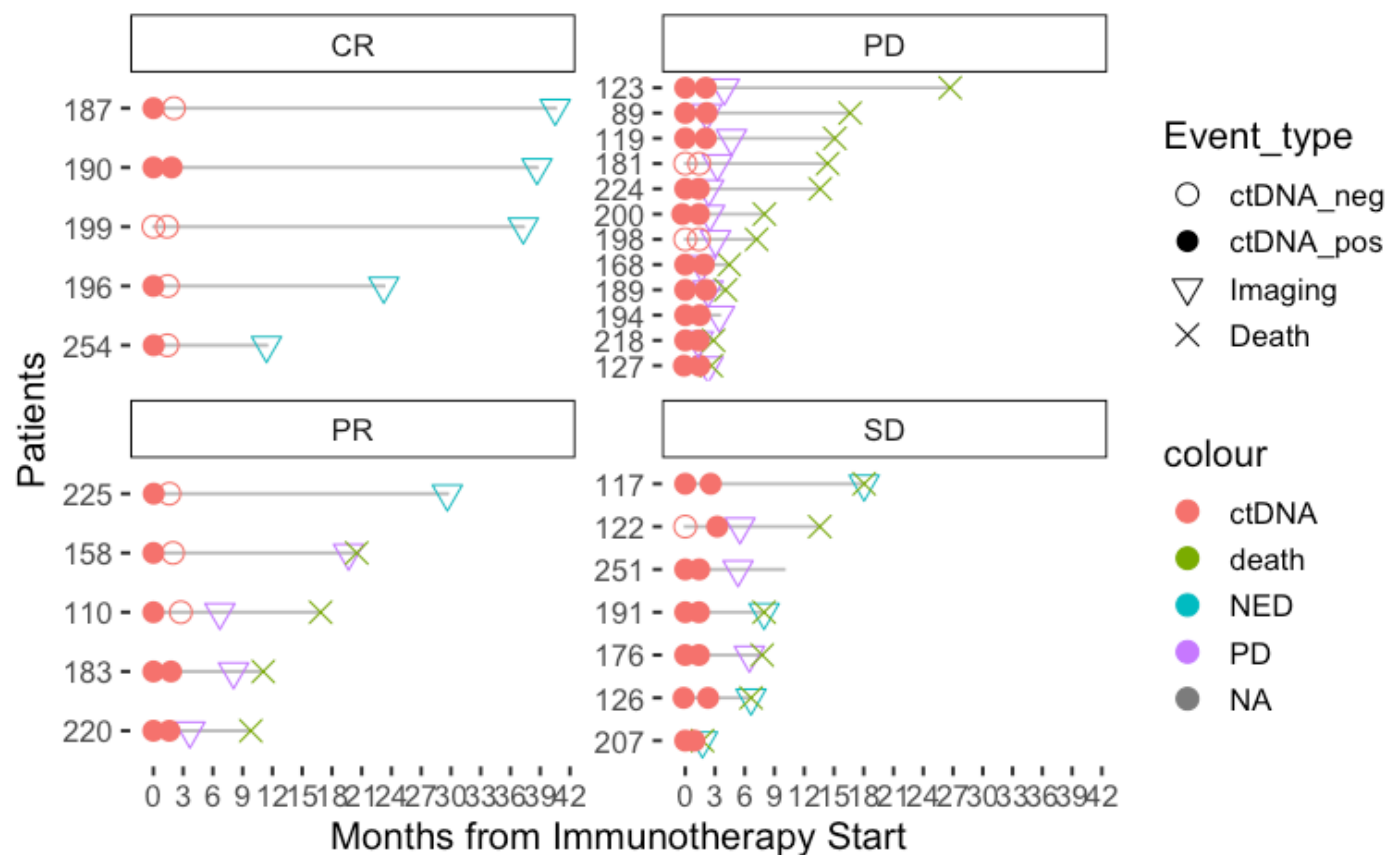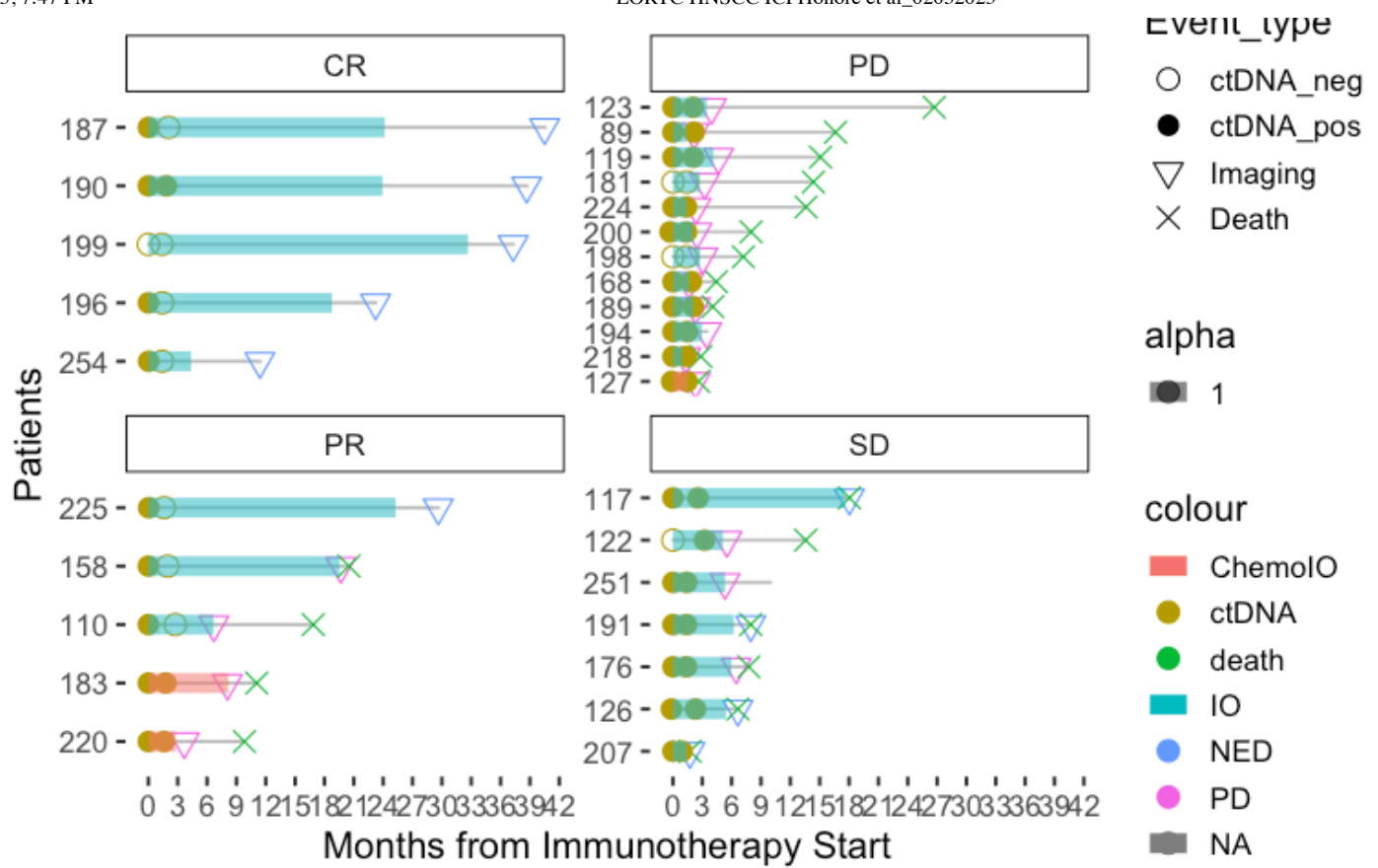
```
                              oplot_ev2 <- oplot_ev1.1 + swimmer_lines(df_lines=cl
instage_df,
                                                                    id='Patient
Name',
                                                                    start='Tx_s
tart.months',
                                                                    end='Tx_en
d.months',
                                                                    name_col='T
x_type',
                                                                    size=3.5,
                                                                    name_alpha
= 1.0)
                              oplot_ev2 <- oplot_ev2 + guides(linetype = guide_leg
end(override.aes = list(size = 5, color = "black")))
                              oplot_ev2
```
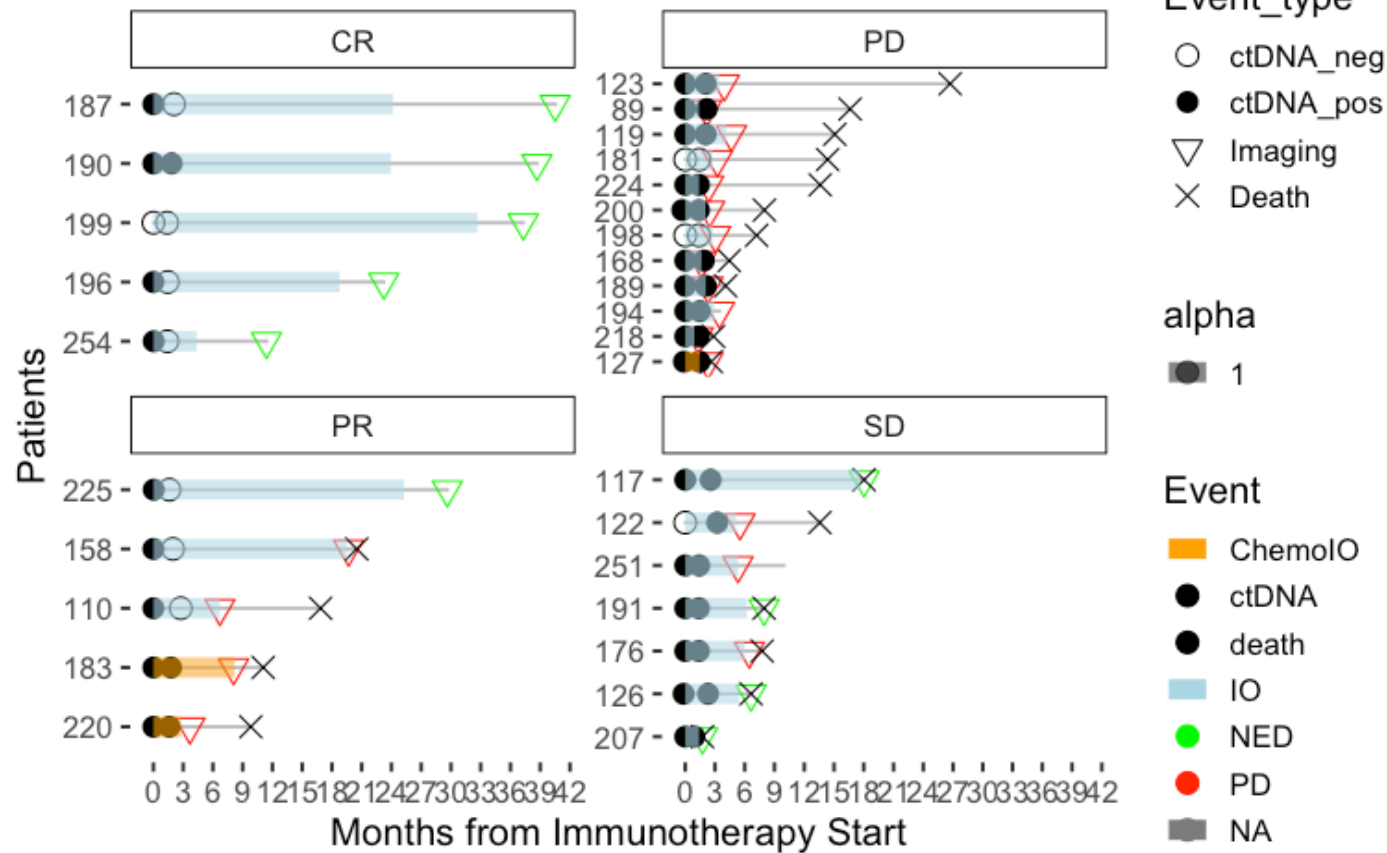
```
oplot_ev2.2 <- oplot_ev2 + ggplot2::scale_color_manual(name="Event",values=c( "orange",
"black", "black", "lightblue", "green", "red"))
oplot_ev2.2
```

#Association of Baseline ctDNA MTM levels with clinicopathological factors

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_datadf <- as.data.frame(circ_data)

tally(~cT.Status, data=circ_data, margins = TRUE)
```
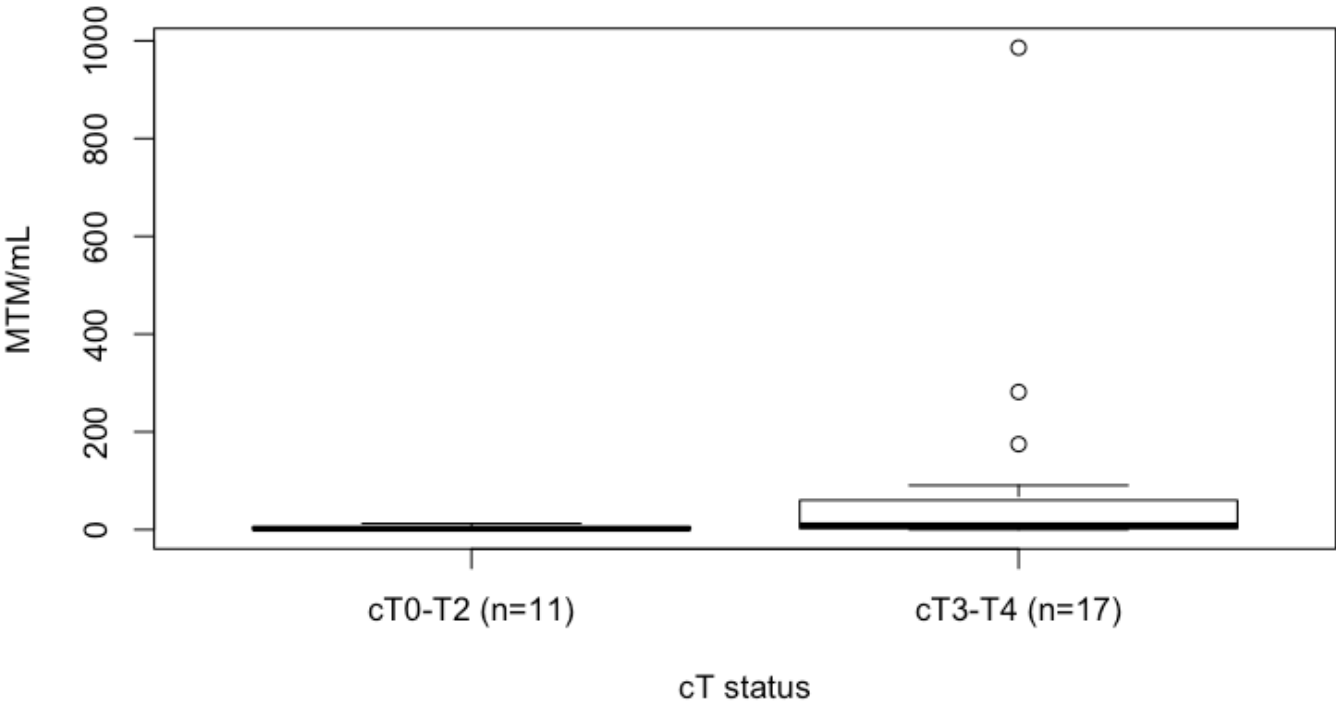
```
cT.Status
cT0-T2 cT3-T4    cTx  Total
    11     17      1     29
```

Hide

```
circ_data$cT.Status <- factor(circ_data$cT.Status, levels = c("cT0-T2","cT3-T4"), labels
= c("cT0-T2 (n=11)","cT3-T4 (n=17)"))
boxplot(ctDNA.Base.MTM~cT.Status, data=circ_data, main="ctDNA pre-treatment MTM - cT sta
tus", xlab="cT status", ylab="MTM/mL", col="white",border="black")
```

ctDNA pre-treatment MTM - cT status

Hide

```
m3<-wilcox.test(ctDNA.Base.MTM ~ cT.Status, data=circ_data, na.rm=TRUE, exact=FALSE, con
f.int=TRUE)
print(m3)
```

```
    Wilcoxon rank sum test with continuity correction

data:  ctDNA.Base.MTM by cT.Status
W = 45.5, p-value = 0.02523
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -57.0000356  -0.7999756
sample estimates:
difference in location
          -7.400021
```

Hide

```
tally(~cN.Status, data=circ_data, margins = TRUE)
```
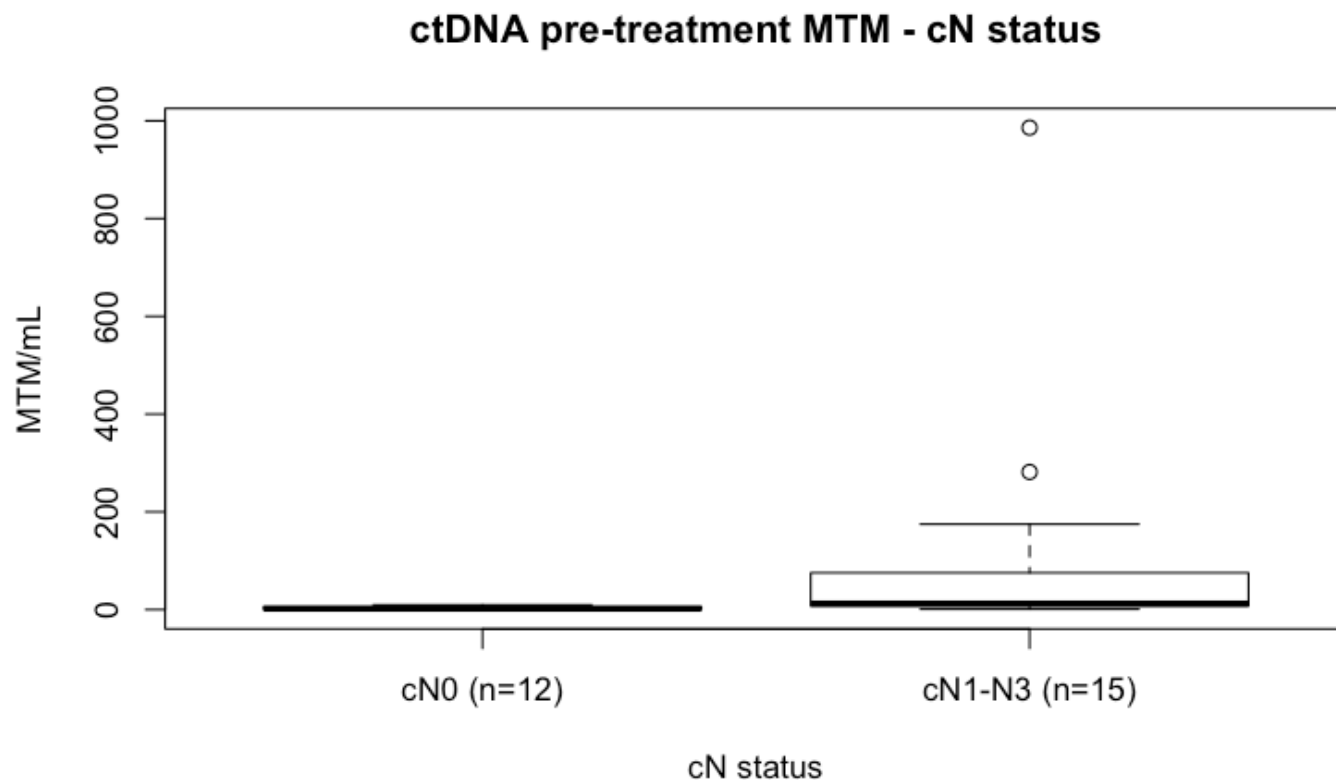
```
cN.Status
   cN0 cN1-N3   cNx  Total
    12     15     2     29
```

Hide

```
circ_data$cN.Status <- factor(circ_data$cN.Status, levels = c("cN0","cN1–N3"), labels =
c("cN0 (n=12)","cN1–N3 (n=15)"))
boxplot(ctDNA.Base.MTM~cN.Status, data=circ_data, main="ctDNA pre-treatment MTM – cN sta
tus", xlab="cN status", ylab="MTM/mL", col="white",border="black")
```

ctDNA pre-treatment MTM - cN status

Hide

```
m4<-wilcox.test(ctDNA.Base.MTM ~ cN.Status, data=circ_data, na.rm=TRUE, exact=FALSE, con
f.int=TRUE)
print(m4)
```

```
    Wilcoxon rank sum test with continuity correction

data:  ctDNA.Base.MTM by cN.Status
W = 32.5, p-value = 0.005379
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -60.200006  -3.299997
sample estimates:
difference in location
            -10.15193
```

#Median MTM/mL levels for ctDNA positive pts pre-treatment

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]

median_ctDNA <- median(circ_data$ctDNA.Base.MTM, na.rm = TRUE)
range_ctDNA <- range(circ_data$ctDNA.Base.MTM, na.rm = TRUE)
cat("Median MTM/mL post-treatment:", median_ctDNA, "\n")
```

```
Median MTM/mL post-treatment: 8.4
```

```
cat("Range MTM/mL post-treatment:", range_ctDNA[1], "-", range_ctDNA[2], "\n")
```

```
Range MTM/mL post-treatment: 0.2 - 986
```

#Median MTM/mL levels for ctDNA positive pts post-treatment

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.postTx!="",]
circ_data <- circ_data[circ_data$ctDNA.postTx=="POSITIVE",]

median_ctDNA <- median(circ_data$ctDNA.postTx.MTM, na.rm = TRUE)
range_ctDNA <- range(circ_data$ctDNA.postTx.MTM, na.rm = TRUE)
cat("Median MTM/mL post-treatment:", median_ctDNA, "\n")
```

```
Median MTM/mL post-treatment: 7.9
```

```
cat("Range MTM/mL post-treatment:", range_ctDNA[1], "-", range_ctDNA[2], "\n")
```

```
Range MTM/mL post-treatment: 0.1 - 737.8
```

#Median time from end treatment to progression for ctDNA negative pts post-treatment

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.postTx!="",]
circ_data <- circ_data[circ_data$ctDNA.postTx=="NEGATIVE",]
circ_data <- circ_data[circ_data$PFS.Event=="TRUE",]

median_PFS <- median(circ_data$PFS.months, na.rm = TRUE)
range_PFS <- range(circ_data$PFS.months, na.rm = TRUE)
cat("Median PFS:", median_PFS, "\n")
```

```
Median PFS: 4.977494
```

Hide

```
cat("Range PFS:", range_PFS[1], "-", range_PFS[2], "\n")
```

```
Range PFS: 3.055492 - 19.67999
```

#PFS by ctDNA status post/during-ICI

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.postTx!="",]
circ_datadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~ctDNA.postTx, dat
a = circ_data)
```

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

                       n events median 0.95LCL 0.95UCL
ctDNA.postTx=NEGATIVE  9      4     NA    6.70      NA
ctDNA.postTx=POSITIVE 20     19   3.81    2.37    6.64
```
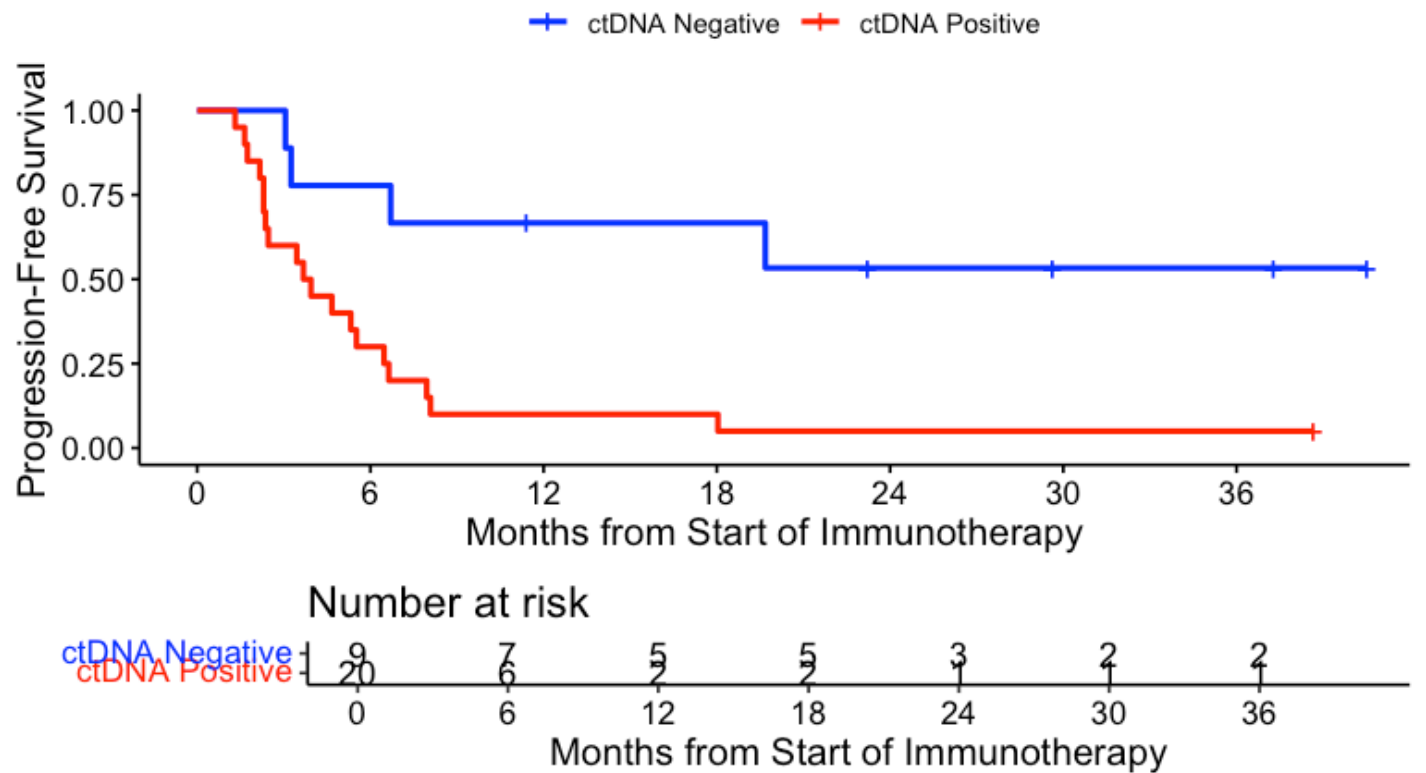
Hide

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

ctDNA.postTx	Total	Events	Fraction	Percentage
<chr>	<int>	<int>	<dbl>	<dbl>
NEGATIVE	9	4	0.4444444	44.44444
POSITIVE	20	19	0.9500000	95.00000

2 rows

Hide

```
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.postTx, data = circ_data,conf.int=0.95,conf.type
="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRU
E, break.time.by=6, palette=c("blue","red"), title="PFS – ctDNA Post/Under treatment", y
lab= "Progression-Free Survival", xlab="Months from Start of Immunotherapy", legend.labs
=c("ctDNA Negative", "ctDNA Positive"), legend.title="")
```

PFS - ctDNA Post/Under treatment


```
summary(KM_curve, times= c(0, 12, 24, 36))
```
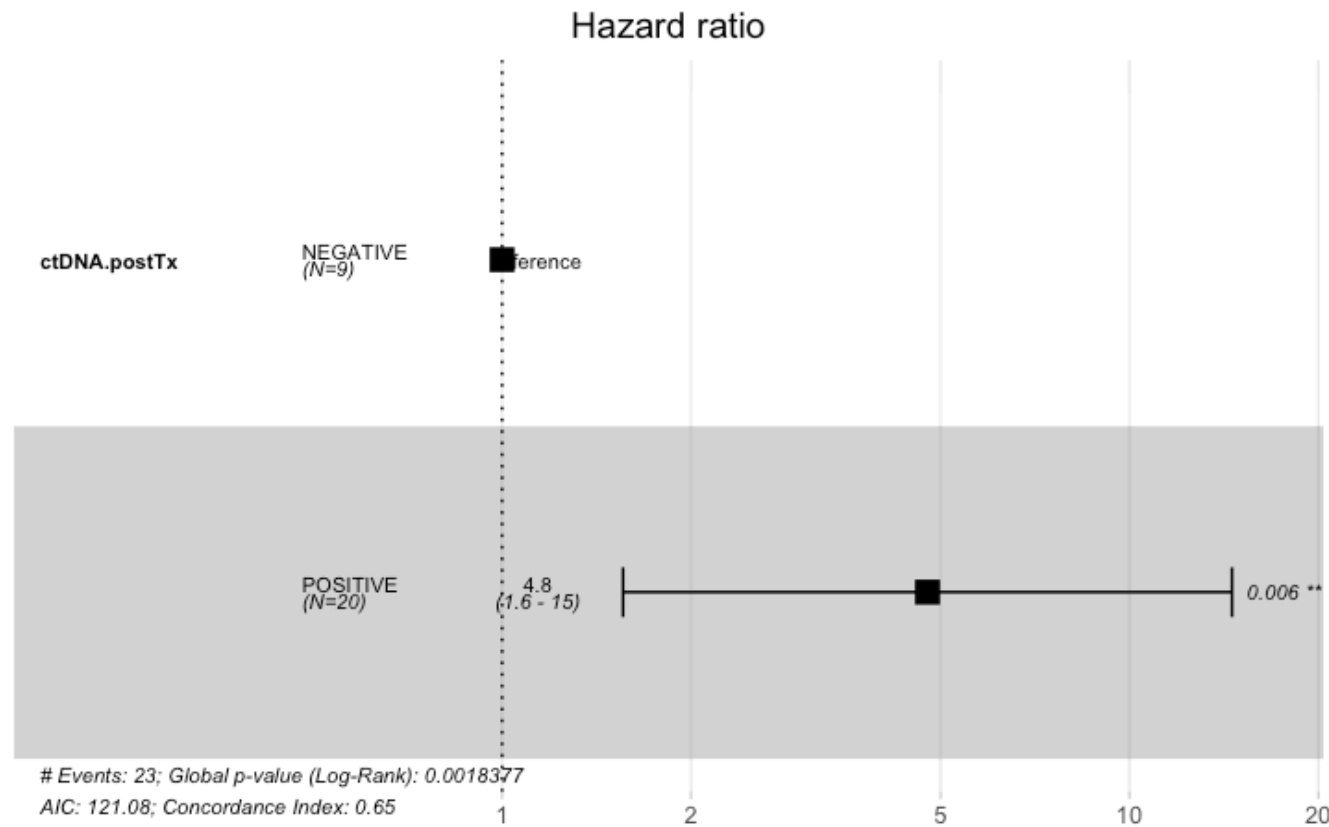
```
Call: survfit(formula = surv_object ~ ctDNA.postTx, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

                ctDNA.postTx=NEGATIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0      9       0    1.000   0.000        1.000        1.000
   12      5       3    0.667   0.157        0.282        0.878
   24      3       1    0.533   0.173        0.177        0.796
   36      2       0    0.533   0.173        0.177        0.796

                ctDNA.postTx=POSITIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0     20       0     1.00  0.0000      1.00000        1.000
   12      2      18     0.10  0.0671      0.01698        0.272
   24      1       1     0.05  0.0487      0.00345        0.205
   36      1       0     0.05  0.0487      0.00345        0.205
```

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

Hazard ratio

ctDNA.postTx   NEGATIVE (N=9)   Reference

POSITIVE (N=20)   4.8 (1.6 - 15)   0.006 **

Events: 23; Global p-value (Log-Rank): 0.0018377
AIC: 121.08; Concordance Index: 0.65

Hide

```
summary(cox_fit)
```

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

  n= 29, number of events= 23

                      coef exp(coef) se(coef)      z Pr(>|z|)
ctDNA.postTxPOSITIVE 1.5616    4.7666   0.5703 2.738  0.00618 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                     exp(coef) exp(-coef) lower .95 upper .95
ctDNA.postTxPOSITIVE     4.767     0.2098     1.559     14.58

Concordance= 0.649  (se = 0.049 )
Likelihood ratio test= 9.7  on 1 df,   p=0.002
Wald test            = 7.5  on 1 df,   p=0.006
Score (logrank) test = 8.78  on 1 df,   p=0.003
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 4.77 (1.56-14.58); p = 0.006"
```

Hide

```
circ_data$ctDNA.postTx <- factor(circ_data$ctDNA.postTx, levels = c("NEGATIVE", "POSITIV
E"), labels = c("Negative", "Positive"))
circ_data$PFS.Event <- factor(circ_data$PFS.Event, levels = c("FALSE", "TRUE"), labels =
c("No Progression", "Progression"))
contingency_table <- table(circ_data$ctDNA.postTx, circ_data$PFS.Event)
chi_square_test <- chisq.test(contingency_table)
```

```
Warning: Chi-squared approximation may be incorrect
```

Hide

```
print(chi_square_test)
```

```
    Pearson's Chi-squared test with Yates' continuity correction

data:  contingency_table
X-squared = 6.8323, df = 1, p-value = 0.008952
```

Hide

```
fisher_exact_test <- fisher.test(contingency_table)
print(fisher_exact_test)
```

```
    Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.005482
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
    1.68957 1169.09723
sample estimates:
odds ratio
  20.33413
```
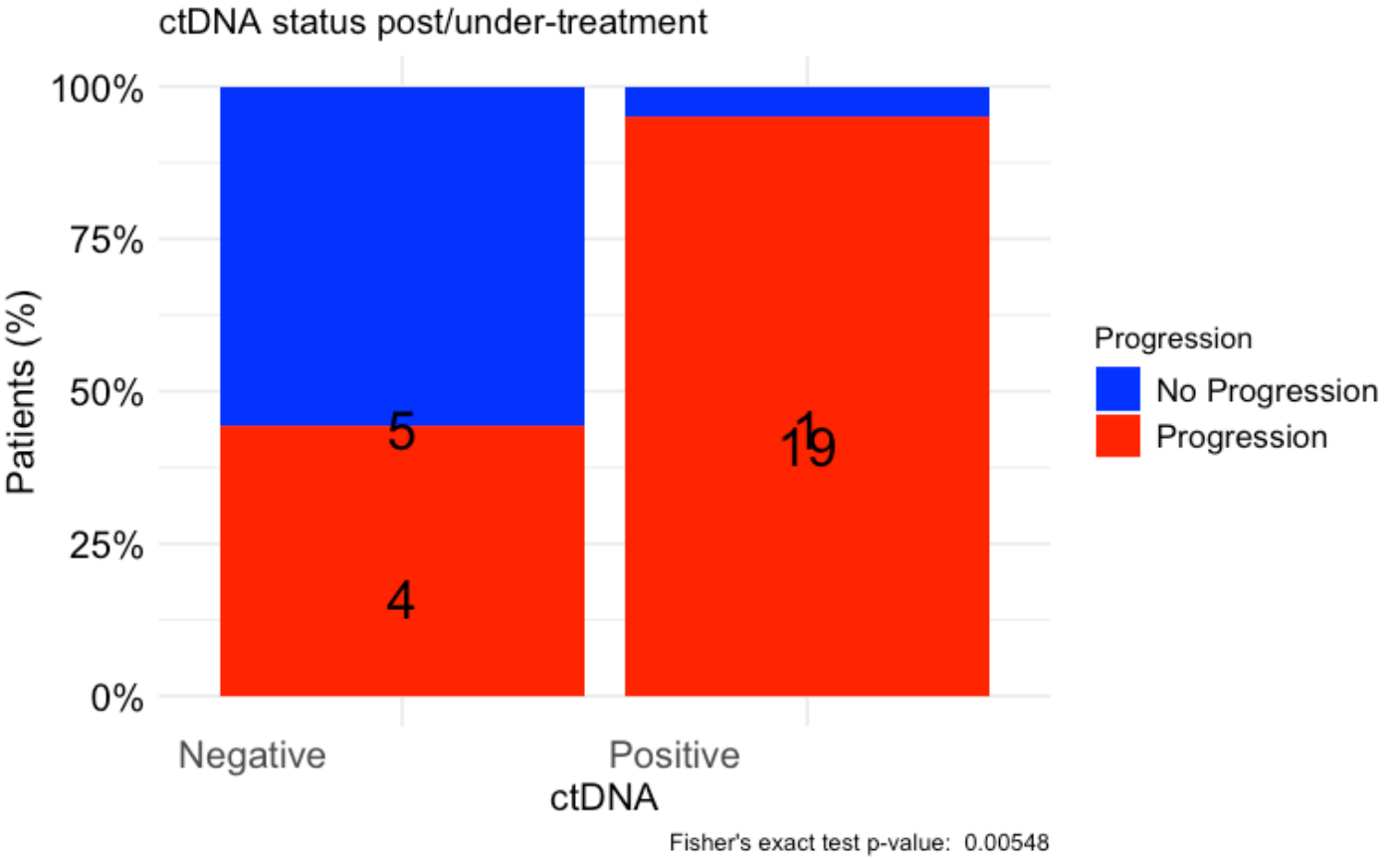
Hide

```
print(contingency_table)
```

```
          No Progression Progression
  Negative              5           4
  Positive              1          19
```

Hide

```
table_df <- as.data.frame(contingency_table)
table_df$Total <- ave(table_df$Freq, table_df$Var1, FUN = sum)
table_df$Percentage <- table_df$Freq / table_df$Total
table_df$MiddlePercentage <- table_df$Percentage / 2
ggplot(table_df, aes(x = Var1, y = Percentage, fill = Var2)) +
  geom_bar(stat = "identity") +
  geom_text(aes(y = MiddlePercentage, label = Freq), position = "stack", color = "blac
k", vjust = 1.5, size = 7) +
  theme_minimal() +
  labs(title = "ctDNA status post/under-treatment",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "Progression",
       caption = paste("Fisher's exact test p-value: ", format.pval(fisher_exact_test$p.
value))) +
  scale_y_continuous(labels = scales::percent_format()) +
  scale_fill_manual(values = c("No Progression" = "blue", "Progression" = "red")) + # de
fine custom colors
  theme(axis.text.x = element_text(angle = 0, hjust = 1.5, size = 14), # increase x-axis
text size
        axis.text.y = element_text(size = 14, color = "black"), # increase y-axis text s
ize
        axis.title.x = element_text(size = 14, color = "black"), # increase x-axis label
size
        axis.title.y = element_text(size = 14, color = "black"), # increase y-axis label
size
        legend.text = element_text(size = 12, color = "black"))  # increase Progression
label size
```

ctDNA status post/under-treatment

Fisher's exact test p-value: 0.00548

#OS by ctDNA status post/during-ICI

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.postTx!="",]
circ_datadf <- as.data.frame(circ_data)

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

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

                        n events median 0.95LCL 0.95UCL
ctDNA.postTx=NEGATIVE  9     4     NA    16.85      NA
ctDNA.postTx=POSITIVE 20    17   10.4    7.75      18
```
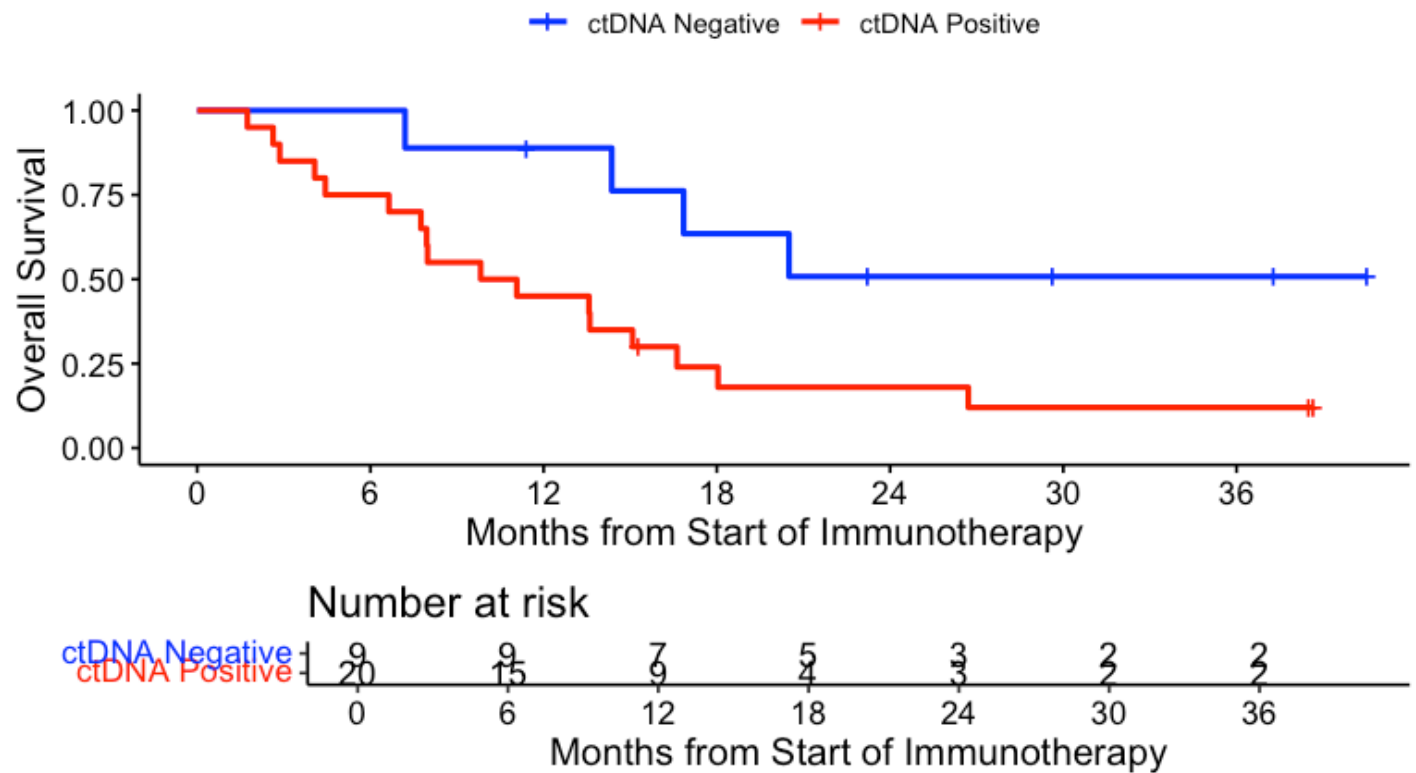
Hide

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

ctDNA.postTx	Total	Events	Fraction	Percentage
<chr>	<int>	<int>	<dbl>	<dbl>
NEGATIVE	9	4	0.4444444	44.44444
POSITIVE	20	17	0.8500000	85.00000

2 rows

Hide

```
surv_object <-Surv(time = circ_data$OS.months, event = circ_data$OS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.postTx, data = circ_data,conf.int=0.95,conf.type
="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRU
E, break.time.by=6, palette=c("blue","red"), title="OS - ctDNA Post/Under treatment", yl
ab= "Overall Survival", xlab="Months from Start of Immunotherapy", legend.labs=c("ctDNA
Negative", "ctDNA Positive"), legend.title="")
```

OS - ctDNA Post/Under treatment


```
summary(KM_curve, times= c(12, 24, 36))
```
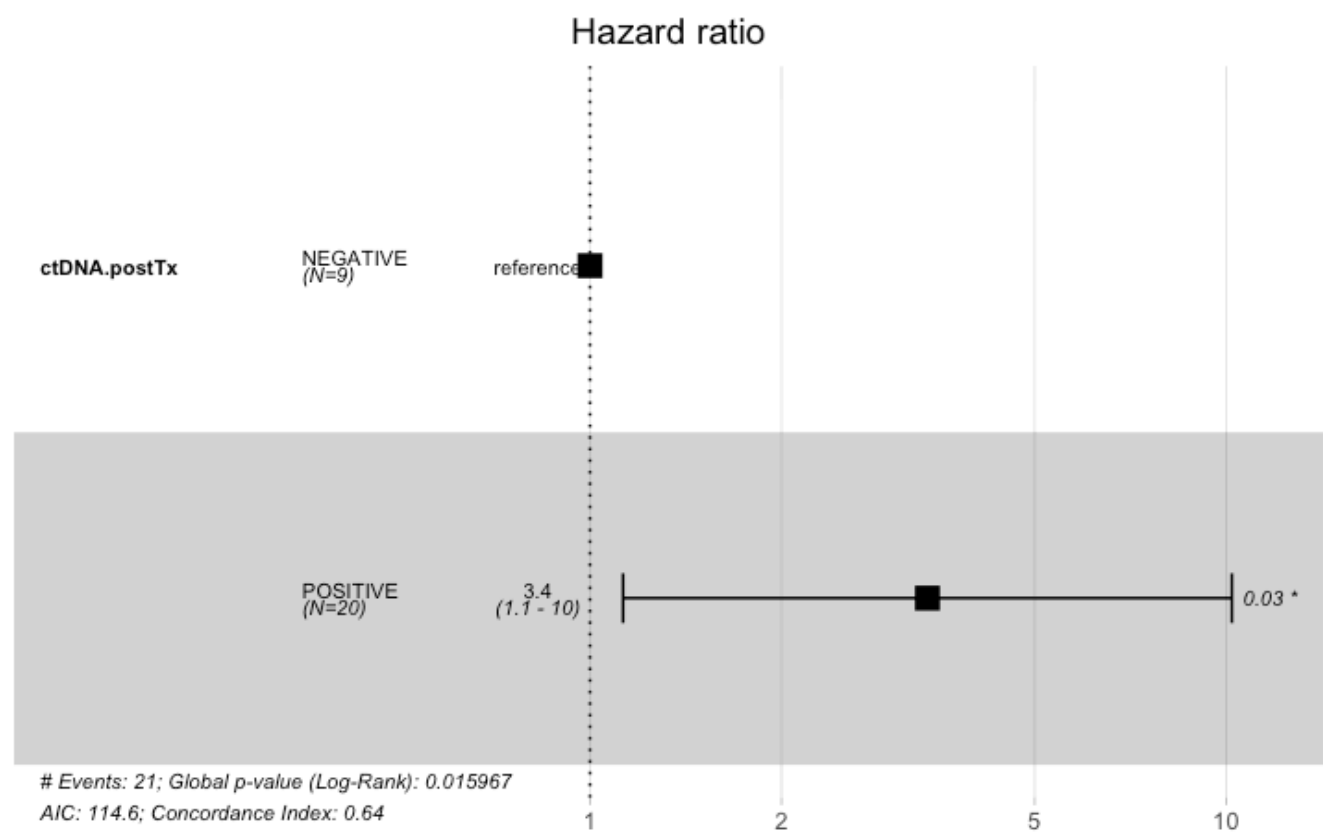
```
Call: survfit(formula = surv_object ~ ctDNA.postTx, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

                ctDNA.postTx=NEGATIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
   12      7       1    0.889   0.105        0.433        0.984
   24      3       3    0.508   0.177        0.157        0.781
   36      2       0    0.508   0.177        0.157        0.781

                ctDNA.postTx=POSITIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
   12      9      11     0.45  0.1112       0.2311        0.647
   24      3       5     0.18  0.0900       0.0480        0.380
   36      2       1     0.12  0.0775       0.0213        0.311
```

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

Hazard ratio


```
ctDNA.postTx    NEGATIVE        reference
                (N=9)

                POSITIVE        3.4                                            0.03 *
                (N=20)          (1.1 - 10)
```

Events: 21; Global p-value (Log-Rank): 0.015967
AIC: 114.6; Concordance Index: 0.64

```
1          2          5          10
```

Hide

```
summary(cox_fit)
```

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

  n= 29, number of events= 21

                        coef exp(coef) se(coef)      z Pr(>|z|)
ctDNA.postTxPOSITIVE 1.2213    3.3918   0.5621 2.173   0.0298 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                     exp(coef) exp(-coef) lower .95 upper .95
ctDNA.postTxPOSITIVE     3.392     0.2948     1.127     10.21

Concordance= 0.638  (se = 0.048 )
Likelihood ratio test= 5.81  on 1 df,    p=0.02
Wald test            = 4.72  on 1 df,    p=0.03
Score (logrank) test = 5.29  on 1 df,    p=0.02
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 3.39 (1.13-10.21); p = 0.03"
```

Hide

```
circ_data$ctDNA.postTx <- factor(circ_data$ctDNA.postTx, levels = c("NEGATIVE", "POSITIV
E"), labels = c("Negative", "Positive"))
circ_data$OS.Event <- factor(circ_data$OS.Event, levels = c("FALSE", "TRUE"), labels = c
("Alive", "Deceased"))
contingency_table <- table(circ_data$ctDNA.postTx, circ_data$OS.Event)
chi_square_test <- chisq.test(contingency_table)
```

```
Warning: Chi-squared approximation may be incorrect
```

Hide

```
print(chi_square_test)
```

```
	Pearson's Chi-squared test with Yates' continuity correction

data:  contingency_table
X-squared = 3.2819, df = 1, p-value = 0.07005
```

Hide

```
fisher_exact_test <- fisher.test(contingency_table)
print(fisher_exact_test)
```

```
    Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.0667
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.8624077 62.5051555
sample estimates:
odds ratio
  6.505452
```
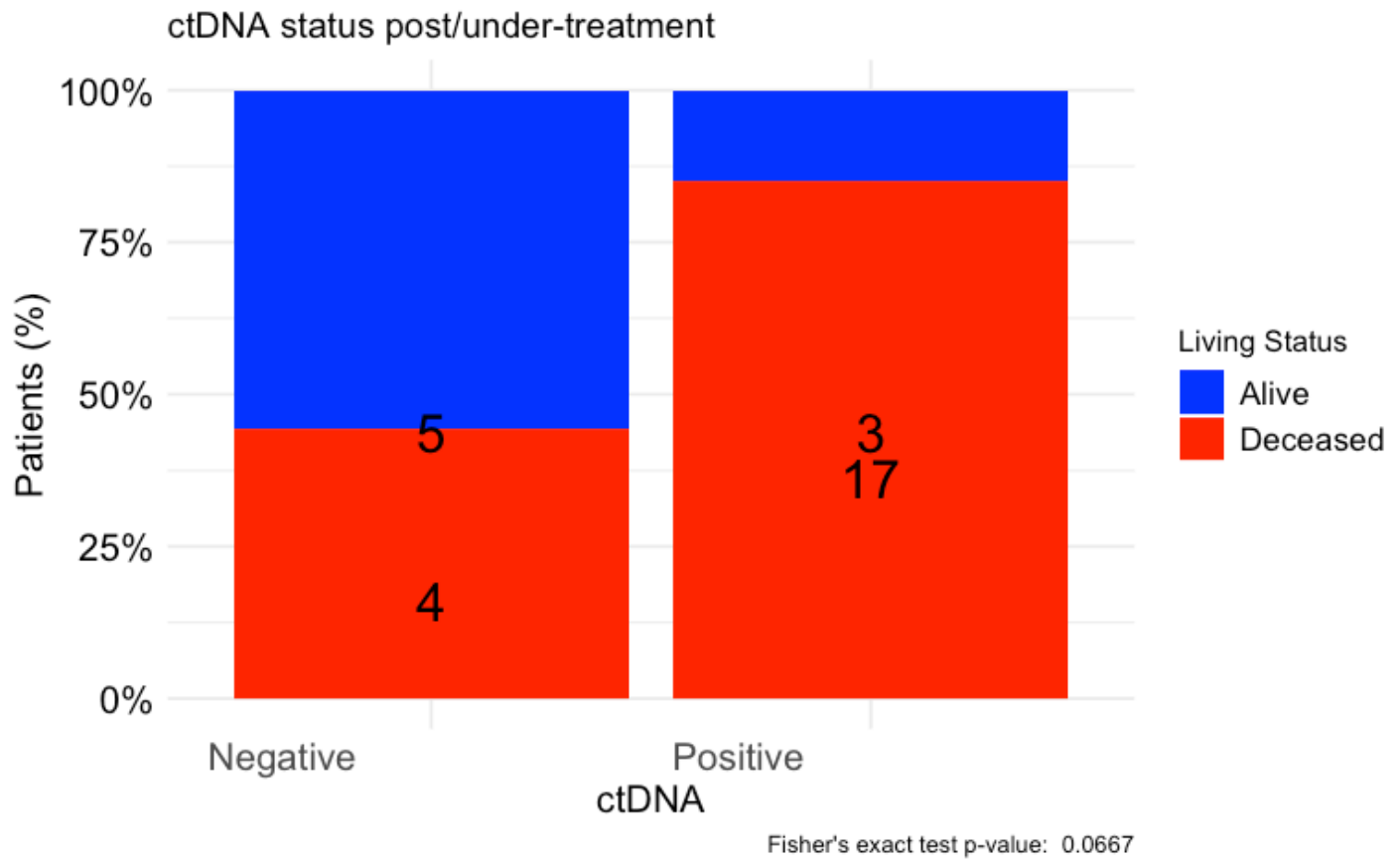
Hide

```
print(contingency_table)
```

```
        Alive Deceased
  Negative   5      4
  Positive   3     17
```

Hide

```r
table_df <- as.data.frame(contingency_table)
table_df$Total <- ave(table_df$Freq, table_df$Var1, FUN = sum)
table_df$Percentage <- table_df$Freq / table_df$Total
table_df$MiddlePercentage <- table_df$Percentage / 2
ggplot(table_df, aes(x = Var1, y = Percentage, fill = Var2)) +
  geom_bar(stat = "identity") +
  geom_text(aes(y = MiddlePercentage, label = Freq), position = "stack", color = "blac
k", vjust = 1.5, size = 7) +
  theme_minimal() +
  labs(title = "ctDNA status post/under-treatment",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "Living Status",
       caption = paste("Fisher's exact test p-value: ", format.pval(fisher_exact_test$p.
value))) +
  scale_y_continuous(labels = scales::percent_format()) +
  scale_fill_manual(values = c("Alive" = "blue", "Deceased" = "red")) + # define custom
colors
  theme(axis.text.x = element_text(angle = 0, hjust = 1.5, size = 14), # increase x-axis
text size
        axis.text.y = element_text(size = 14, color = "black"), # increase y-axis text s
ize
        axis.title.x = element_text(size = 14, color = "black"), # increase x-axis label
size
        axis.title.y = element_text(size = 14, color = "black"), # increase y-axis label
size
        legend.text = element_text(size = 12, color = "black"))  # increase Progression
label size
```

ctDNA status post/under-treatment

Fisher's exact test p-value: 0.0667

#Association of ctDNA status post/during-ICI with BOR

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_datadf <- as.data.frame(circ_data)

circ_data$ctDNA.postTx <- factor(circ_data$ctDNA.postTx, levels = c("NEGATIVE", "POSITIV
E"), labels = c("Negative", "Positive"))
circ_data$RESIST <- factor(circ_data$RESIST, levels = c("CR", "PR", "SD", "PD"))
contingency_table <- table(circ_data$ctDNA.postTx, circ_data$RESIST)
chi_square_test <- chisq.test(contingency_table)
```

```
Warning: Chi-squared approximation may be incorrect
```

Hide

```
print(chi_square_test)
```

```
	Pearson's Chi-squared test

data:  contingency_table
X-squared = 11.869, df = 3, p-value = 0.007847
```

Hide

```
fisher_exact_test <- fisher.test(contingency_table)
print(fisher_exact_test)
```

```
	Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.006541
alternative hypothesis: two.sided
```
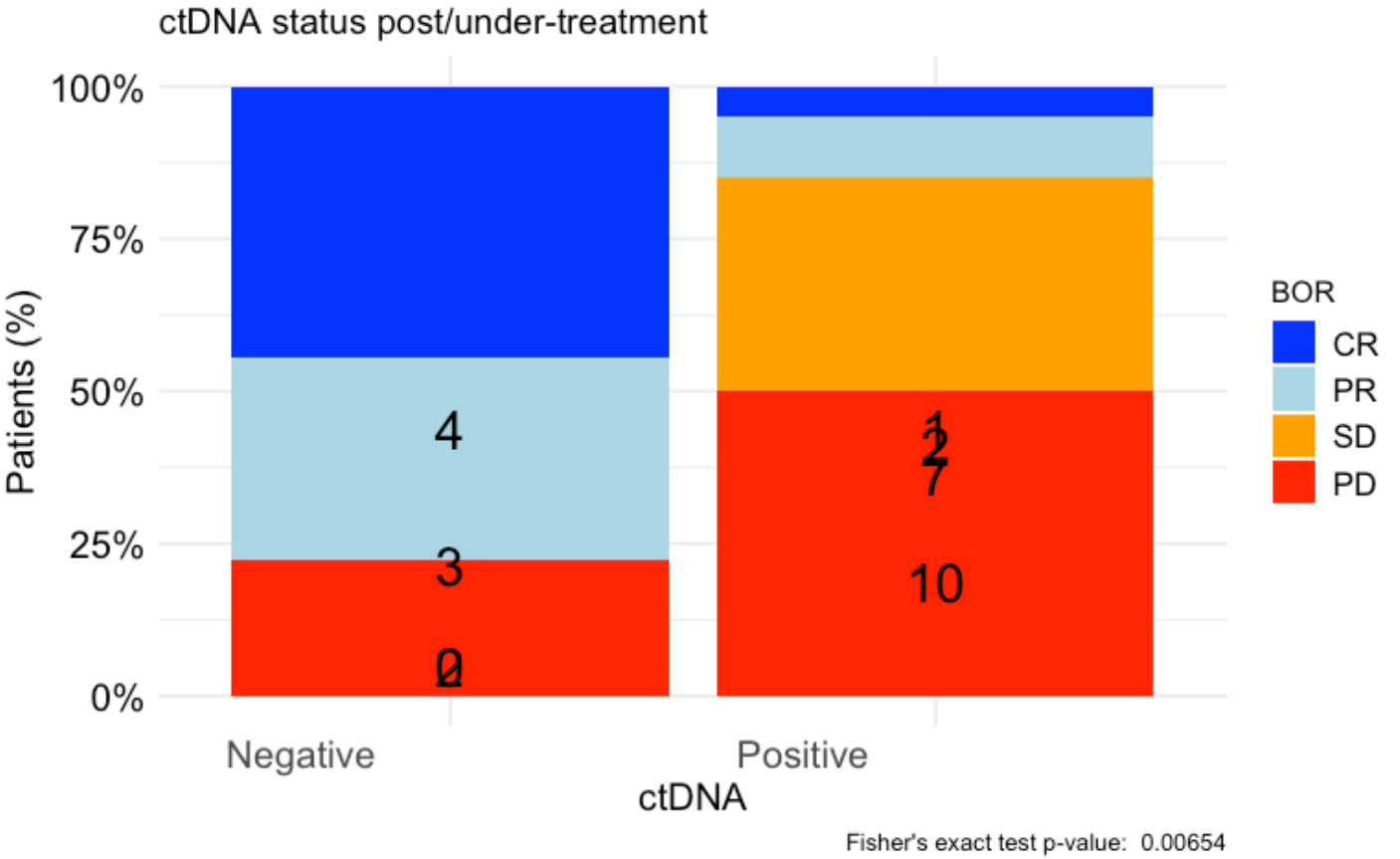
Hide

```
print(contingency_table)
```

```
         CR PR SD PD
  Negative  4  3  0  2
  Positive  1  2  7 10
```

Hide

```r
table_df <- as.data.frame(contingency_table)
table_df$Total <- ave(table_df$Freq, table_df$Var1, FUN = sum)
table_df$Percentage <- table_df$Freq / table_df$Total
table_df$MiddlePercentage <- table_df$Percentage / 2
ggplot(table_df, aes(x = Var1, y = Percentage, fill = Var2)) +
  geom_bar(stat = "identity") +
  geom_text(aes(y = MiddlePercentage, label = Freq), position = "stack", color = "blac
k", vjust = 1.5, size = 7) +
  theme_minimal() +
  labs(title = "ctDNA status post/under-treatment",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "BOR",
       caption = paste("Fisher's exact test p-value: ", format.pval(fisher_exact_test$p.
value))) +
  scale_y_continuous(labels = scales::percent_format()) +
  scale_fill_manual(values = c("CR" = "blue", "PR" = "lightblue", "SD" = "orange", "PD"
= "red")) + # define custom colors
  theme(axis.text.x = element_text(angle = 0, hjust = 1.5, size = 14), # increase x-axis
text size
        axis.text.y = element_text(size = 14, color = "black"), # increase y-axis text s
ize
        axis.title.x = element_text(size = 14, color = "black"), # increase x-axis label
size
        axis.title.y = element_text(size = 14, color = "black"), # increase y-axis label
size
        legend.text = element_text(size = 12, color = "black"))  # increase Progression
label size
```

ctDNA status post/under-treatment

Fisher's exact test p-value: 0.00654

#PFS by ctDNA kinetics post/during-ICI

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ΔctDNA!="",]
circ_datadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~ΔctDNA, data = ci
rc_data)
```

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

                 n events median 0.95LCL 0.95UCL
ΔctDNA=NEGATIVE 13      8  18.04     6.7      NA
ΔctDNA=POSITIVE 12     12   2.41     2.3      NA
```

Hide

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

| ΔctDNA	Total	Events	Fraction	Percentage
<chr>	<int>	<int>	<dbl>	<dbl>
NEGATIVE	13	8	0.6153846	61.53846
POSITIVE	12	12	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 ~ ΔctDNA, data = circ_data,conf.int=0.95,conf.type="log-
log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRU
E, break.time.by=6, palette=c("blue","red"), title="PFS – ctDNA Kinetics Post/Under trea
tment", ylab= "Progression–Free Survival", xlab="Months from Start of Immunotherapy", le
gend.labs=c("Decreased ΔctDNA", "Increased ΔctDNA"), legend.title="")
```

PFS - ctDNA Kinetics Post/Under treatment
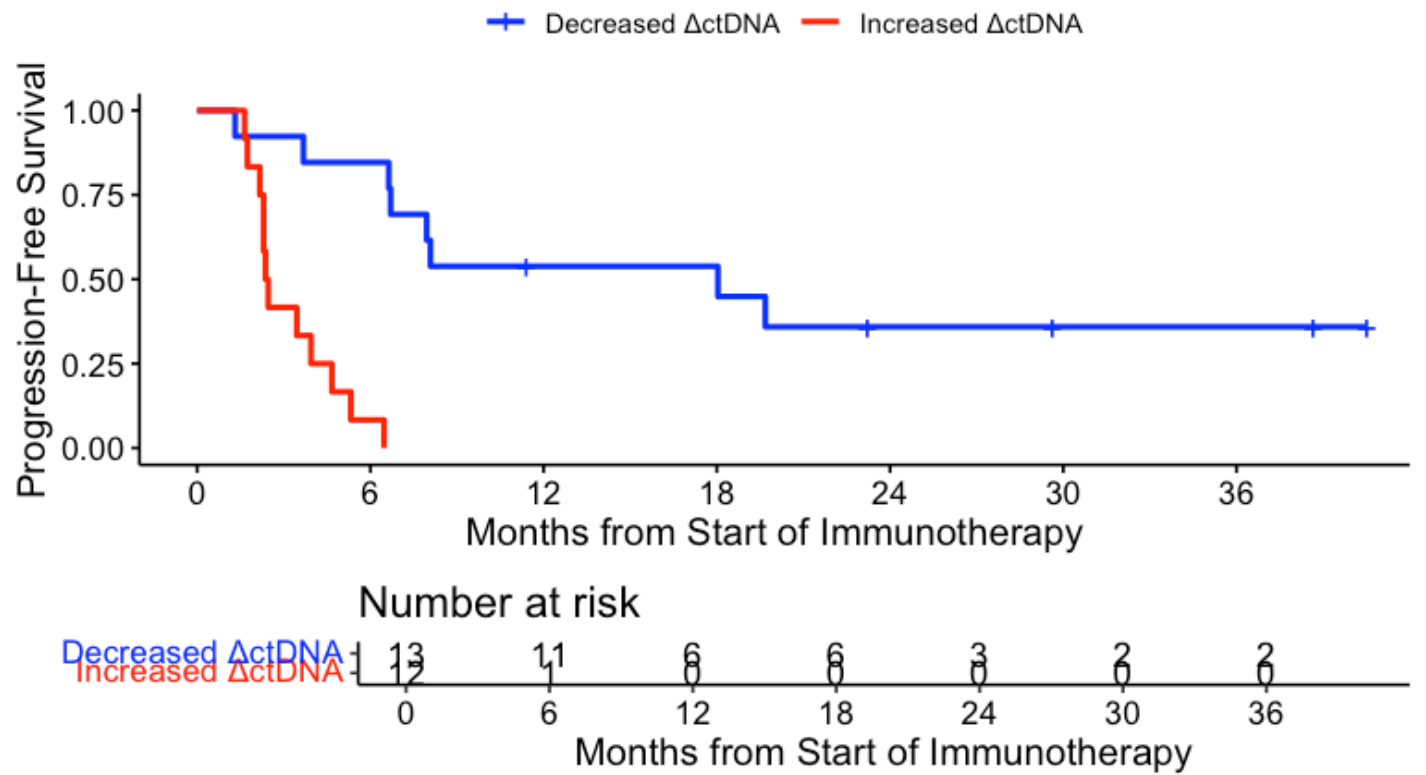
Number at risk

```
summary(KM_curve, times= c(0, 12, 24, 36))
```
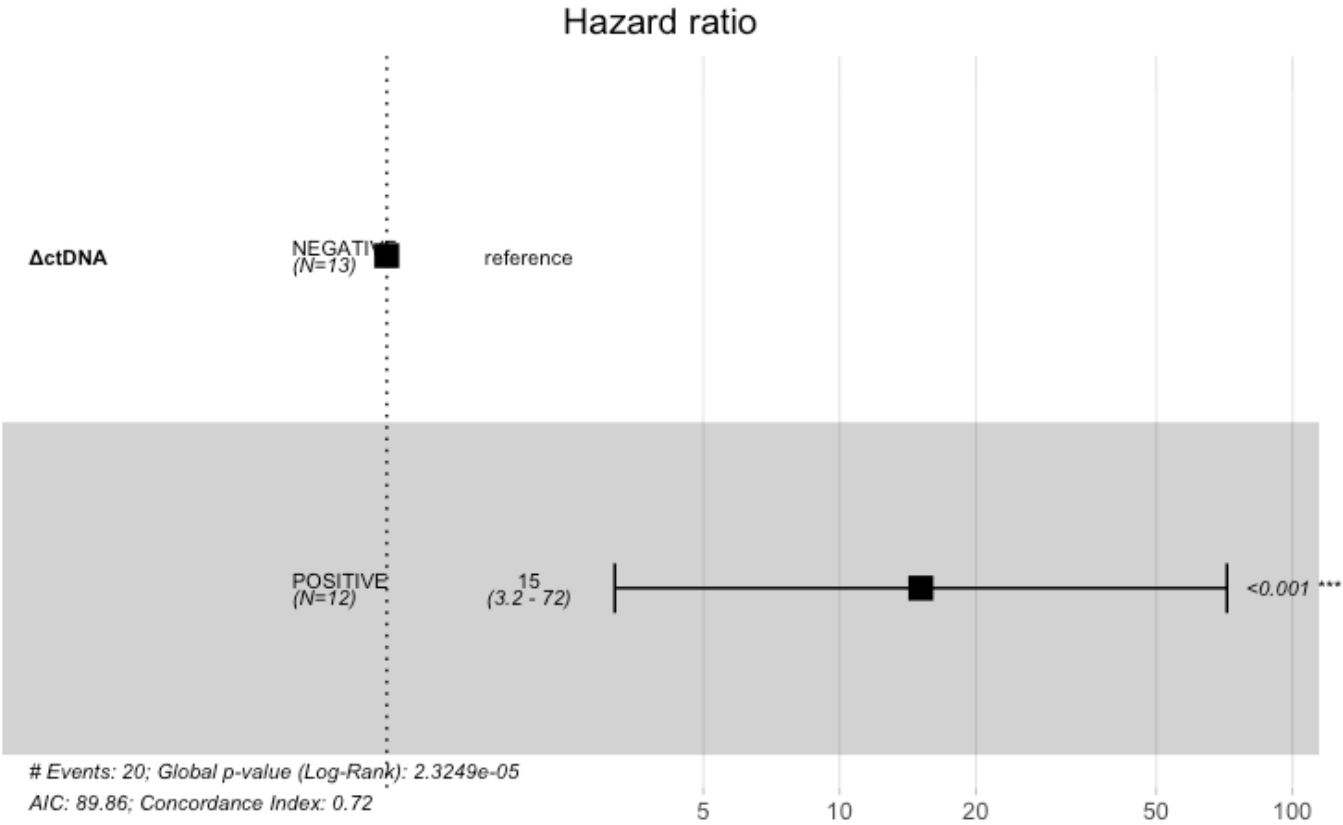
```
Call: survfit(formula = surv_object ~ ΔctDNA, data = circ_data, conf.int = 0.95,
    conf.type = "log-log")
```

                ΔctDNA=NEGATIVE

time	n.risk	n.event	survival	std.err	lower 95% CI	upper 95% CI
0	13	0	1.000	0.000	1.000	1.000
12	6	6	0.538	0.138	0.248	0.760
24	3	2	0.359	0.139	0.117	0.613
36	2	0	0.359	0.139	0.117	0.613

                ΔctDNA=POSITIVE

time	n.risk	n.event	survival	std.err	lower 95% CI	upper 95% CI
0	12	0	1	0	1	1

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

Hazard ratio

ΔctDNA

NEGATIVE
(N=13)   reference

POSITIVE
(N=12)   15
              (3.2 - 72)   <0.001 ***

Events: 20; Global p-value (Log-Rank): 2.3249e-05
AIC: 89.86; Concordance Index: 0.72

5   10   20   50   100

[Hide]

```
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ ΔctDNA, data = circ_data)

  n= 25, number of events= 20

               coef exp(coef) se(coef)    z Pr(>|z|)
ΔctDNAPOSITIVE 2.716    15.112    0.794 3.42 0.000626 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

              exp(coef) exp(-coef) lower .95 upper .95
ΔctDNAPOSITIVE    15.11    0.06617     3.188     71.64

Concordance= 0.716  (se = 0.044 )
Likelihood ratio test= 17.9  on 1 df,   p=2e-05
Wald test            = 11.7  on 1 df,   p=6e-04
Score (logrank) test = 18.33  on 1 df,   p=2e-05
```

[Hide]

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 15.11 (3.19-71.64); p = 0.001"
```

Hide

```
circ_data$ΔctDNA <- factor(circ_data$ΔctDNA, levels = c("NEGATIVE", "POSITIVE"), labels
= c("Decreased", "Increased"))
circ_data$PFS.Event <- factor(circ_data$PFS.Event, levels = c("FALSE", "TRUE"), labels =
c("No Progression", "Progression"))
contingency_table <- table(circ_data$ΔctDNA, circ_data$PFS.Event)
chi_square_test <- chisq.test(contingency_table)
```

```
Warning: Chi-squared approximation may be incorrect
```

Hide

```
print(chi_square_test)
```

```
	Pearson's Chi-squared test with Yates' continuity correction

data:  contingency_table
X-squared = 3.6158, df = 1, p-value = 0.05723
```

Hide

```
fisher_exact_test <- fisher.test(contingency_table)
print(fisher_exact_test)
```

```
    Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.03913
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 1.013281      Inf
sample estimates:
odds ratio
      Inf
```
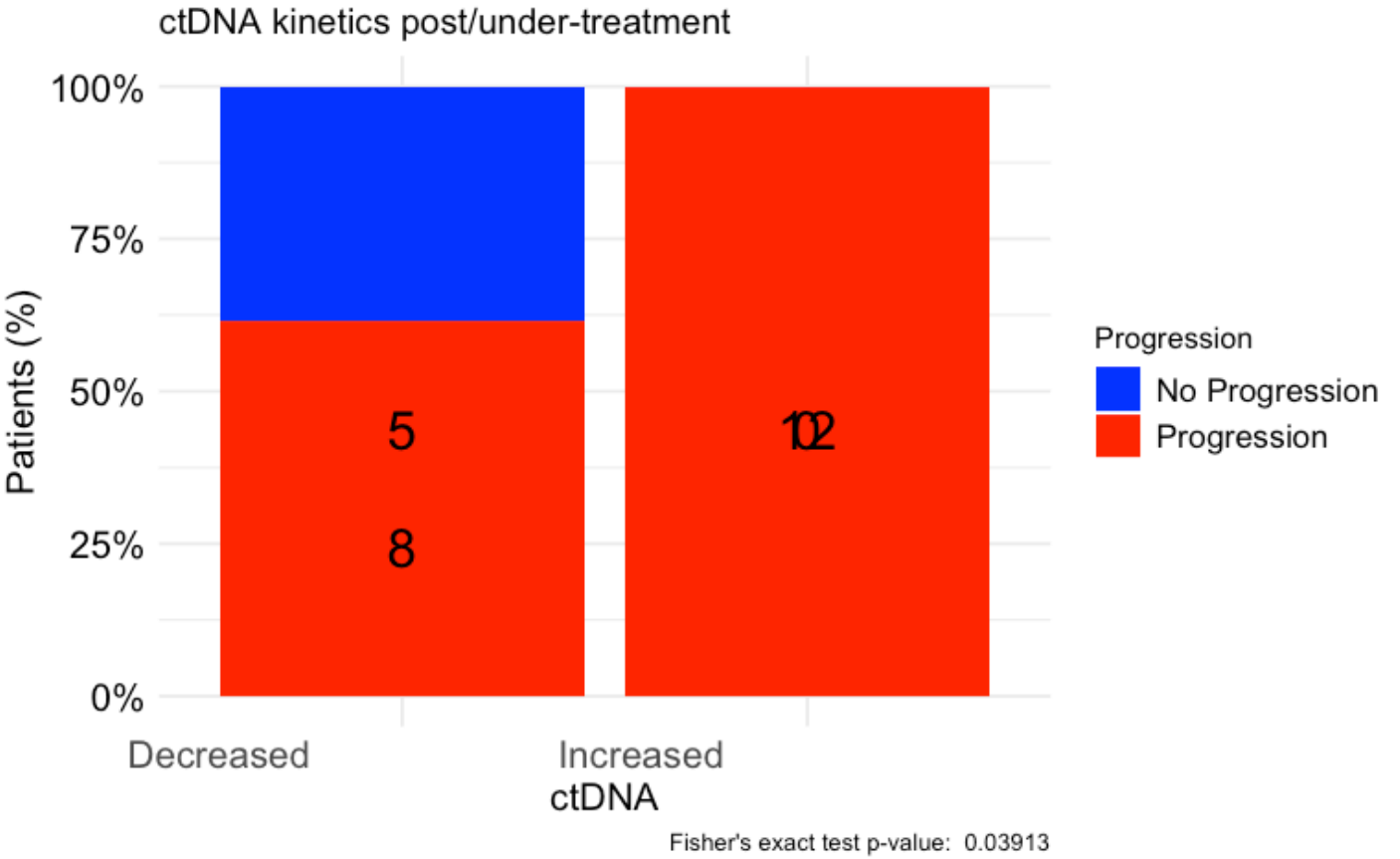
Hide

```
print(contingency_table)
```

```
           No Progression Progression
  Decreased              5           8
  Increased              0          12
```

Hide

```r
table_df <- as.data.frame(contingency_table)
table_df$Total <- ave(table_df$Freq, table_df$Var1, FUN = sum)
table_df$Percentage <- table_df$Freq / table_df$Total
table_df$MiddlePercentage <- table_df$Percentage / 2
ggplot(table_df, aes(x = Var1, y = Percentage, fill = Var2)) +
  geom_bar(stat = "identity") +
  geom_text(aes(y = MiddlePercentage, label = Freq), position = "stack", color = "blac
k", vjust = 1.5, size = 7) +
  theme_minimal() +
  labs(title = "ctDNA kinetics post/under-treatment",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "Progression",
       caption = paste("Fisher's exact test p-value: ", format.pval(fisher_exact_test$p.
value))) +
  scale_y_continuous(labels = scales::percent_format()) +
  scale_fill_manual(values = c("No Progression" = "blue", "Progression" = "red")) + # de
fine custom colors
  theme(axis.text.x = element_text(angle = 0, hjust = 1.5, size = 14), # increase x-axis
text size
        axis.text.y = element_text(size = 14, color = "black"), # increase y-axis text s
ize
        axis.title.x = element_text(size = 14, color = "black"), # increase x-axis label
size
        axis.title.y = element_text(size = 14, color = "black"), # increase y-axis label
size
        legend.text = element_text(size = 12, color = "black"))  # increase Progression
label size
```

ctDNA kinetics post/under-treatment

Fisher's exact test p-value: 0.03913

#OS by ctDNA kinetics post/during-ICI

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ΔctDNA!="",]
circ_datadf <- as.data.frame(circ_data)

survfit(Surv(time = circ_data$OS.months, event = circ_data$OS.Event)~ΔctDNA, data = circ
_data)
```

```
Call: survfit(formula = Surv(time = circ_data$OS.months, event = circ_data$OS.Event) ~
    ΔctDNA, data = circ_data)

                  n events median 0.95LCL 0.95UCL
ΔctDNA=NEGATIVE 13      8   18.0    9.82      NA
ΔctDNA=POSITIVE 12     10   10.8    4.44      NA
```

Hide

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

ΔctDNA	Total	Events	Fraction	Percentage
<chr>	<int>	<int>	<dbl>	<dbl>
NEGATIVE	13	8	0.6153846	61.53846
POSITIVE	12	10	0.8333333	83.33333

2 rows
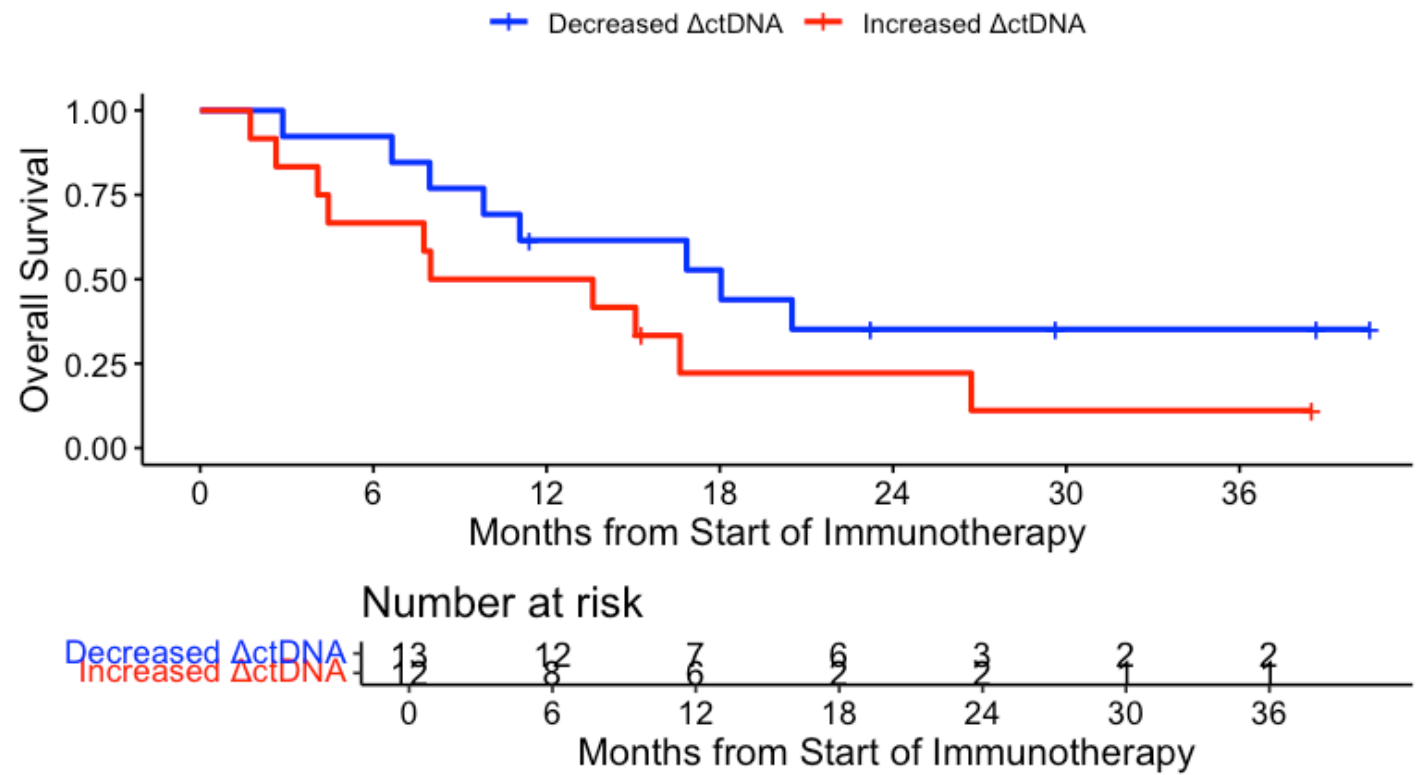
Hide

```
surv_object <-Surv(time = circ_data$OS.months, event = circ_data$OS.Event)
KM_curve <- survfit(surv_object ~ ΔctDNA, data = circ_data,conf.int=0.95,conf.type="log-
log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRU
E, break.time.by=6, palette=c("blue","red"), title="OS – ctDNA Kinetics Post/Under treat
ment", ylab= "Overall Survival", xlab="Months from Start of Immunotherapy", legend.labs=
c("Decreased ΔctDNA", "Increased ΔctDNA"), legend.title="")
```

OS - ctDNA Kinetics Post/Under treatment

Number at risk

```
summary(KM_curve, times= c(0, 12, 24, 36))
```
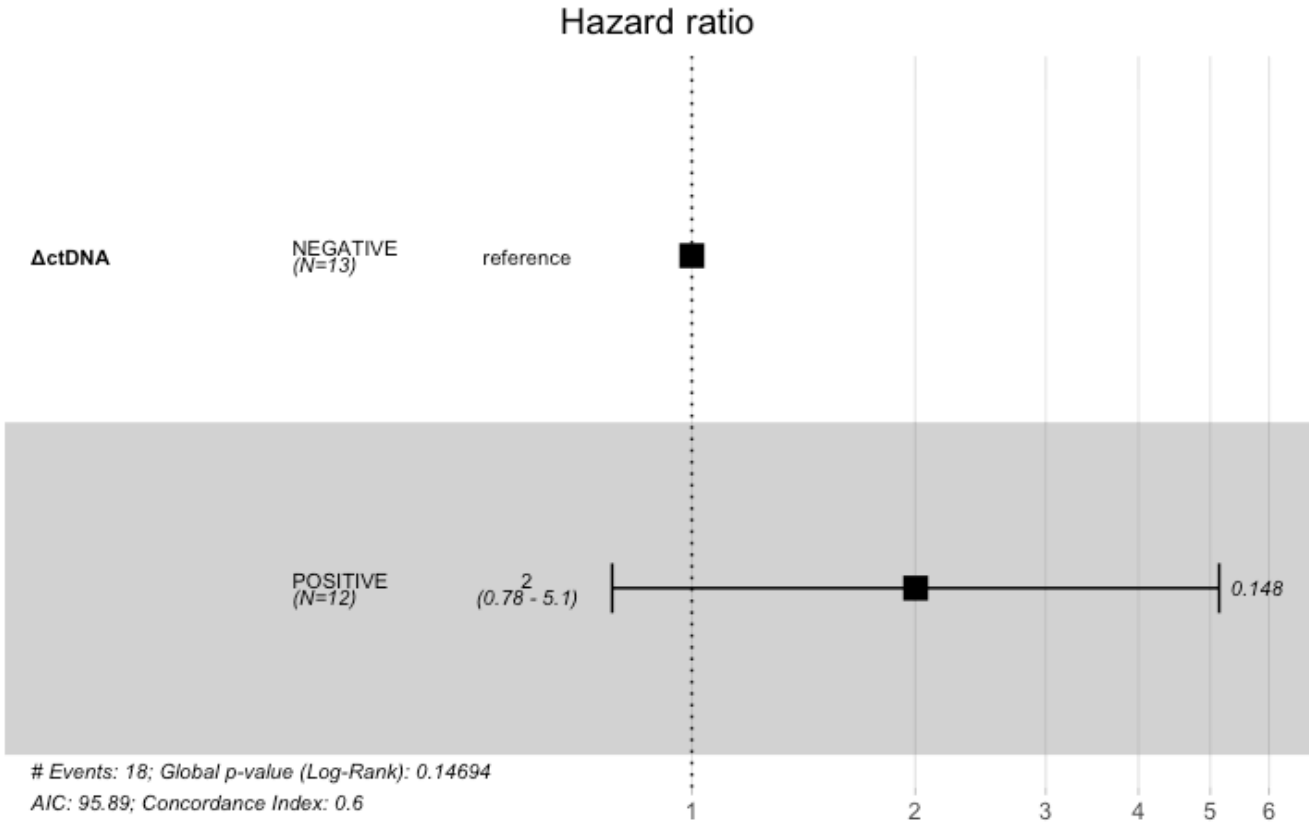
```
Call: survfit(formula = surv_object ~ ΔctDNA, data = circ_data, conf.int = 0.95,
    conf.type = "log-log")

                ΔctDNA=NEGATIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0     13       0    1.000   0.000        1.000        1.000
   12      7       5    0.615   0.135        0.308        0.818
   24      3       3    0.352   0.139        0.112        0.607
   36      2       0    0.352   0.139        0.112        0.607

                ΔctDNA=POSITIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0     12       0    1.000   0.000      1.00000        1.000
   12      6       6    0.500   0.144      0.20848        0.736
   24      2       3    0.222   0.128      0.04111        0.492
   36      1       1    0.111   0.101      0.00701        0.378
```

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

Hazard ratio

ΔctDNA | NEGATIVE (N=13) | reference

POSITIVE (N=12) | 2 (0.78 - 5.1) | 0.148

Events: 18; Global p-value (Log-Rank): 0.14694
AIC: 95.89; Concordance Index: 0.6

```
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ ΔctDNA, data = circ_data)

  n= 25, number of events= 18

                coef exp(coef) se(coef)      z Pr(>|z|)
ΔctDNAPOSITIVE 0.6947    2.0031   0.4806 1.445    0.148

            exp(coef) exp(-coef) lower .95 upper .95
ΔctDNAPOSITIVE    2.003     0.4992    0.7809     5.138

Concordance= 0.598  (se = 0.061 )
Likelihood ratio test= 2.1  on 1 df,   p=0.1
Wald test            = 2.09  on 1 df,   p=0.1
Score (logrank) test = 2.17  on 1 df,   p=0.1
```

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 2 (0.78-5.14); p = 0.148"
```

Hide

```
circ_data$ΔctDNA <- factor(circ_data$ΔctDNA, levels = c("NEGATIVE", "POSITIVE"), labels
= c("Decreased", "Increased"))
circ_data$OS.Event <- factor(circ_data$OS.Event, levels = c("FALSE", "TRUE"), labels = c
("Alive", "Deceased"))
contingency_table <- table(circ_data$ΔctDNA, circ_data$OS.Event)
chi_square_test <- chisq.test(contingency_table)
```

```
Warning: Chi-squared approximation may be incorrect
```

Hide

```
print(chi_square_test)
```

```
	Pearson's Chi-squared test with Yates' continuity correction

data:  contingency_table
X-squared = 0.58792, df = 1, p-value = 0.4432
```

Hide

```
fisher_exact_test <- fisher.test(contingency_table)
print(fisher_exact_test)
```

```
    Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.3783
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.363132 39.390335
sample estimates:
odds ratio
  2.985191
```
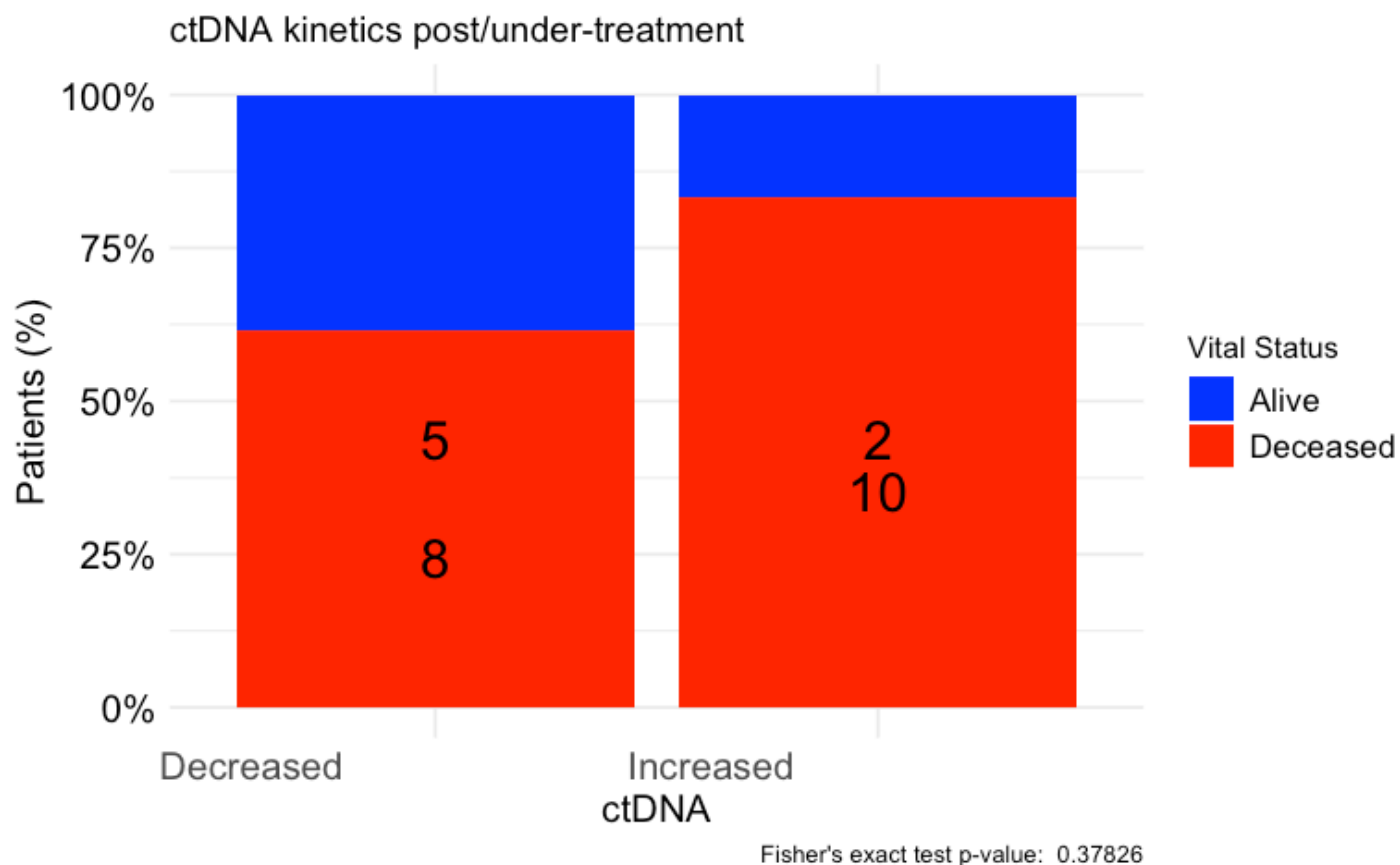
Hide

```
print(contingency_table)
```

```
          Alive Deceased
  Decreased     5        8
  Increased     2       10
```

Hide

```r
table_df <- as.data.frame(contingency_table)
table_df$Total <- ave(table_df$Freq, table_df$Var1, FUN = sum)
table_df$Percentage <- table_df$Freq / table_df$Total
table_df$MiddlePercentage <- table_df$Percentage / 2
ggplot(table_df, aes(x = Var1, y = Percentage, fill = Var2)) +
  geom_bar(stat = "identity") +
  geom_text(aes(y = MiddlePercentage, label = Freq), position = "stack", color = "blac
k", vjust = 1.5, size = 7) +
  theme_minimal() +
  labs(title = "ctDNA kinetics post/under-treatment",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "Vital Status",
       caption = paste("Fisher's exact test p-value: ", format.pval(fisher_exact_test$p.
value))) +
  scale_y_continuous(labels = scales::percent_format()) +
  scale_fill_manual(values = c("Alive" = "blue", "Deceased" = "red")) + # define custom
colors
  theme(axis.text.x = element_text(angle = 0, hjust = 1.5, size = 14), # increase x-axis
text size
        axis.text.y = element_text(size = 14, color = "black"), # increase y-axis text s
ize
        axis.title.x = element_text(size = 14, color = "black"), # increase x-axis label
size
        axis.title.y = element_text(size = 14, color = "black"), # increase y-axis label
size
        legend.text = element_text(size = 12, color = "black"))  # increase Progression
label size
```

ctDNA kinetics post/under-treatment

Fisher's exact test p-value: 0.37826

#Association of ctDNA kinetics post/during-ICI with BOR

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ΔctDNA!="",]
circ_datadf <- as.data.frame(circ_data)

circ_data$ΔctDNA <- factor(circ_data$ΔctDNA, levels = c("NEGATIVE", "POSITIVE"), labels
= c("Decreased", "Increased"))
circ_data$RESIST <- factor(circ_data$RESIST, levels = c("CR", "PR", "SD", "PD"))
contingency_table <- table(circ_data$ΔctDNA, circ_data$RESIST)
chi_square_test <- chisq.test(contingency_table)
```

```
Warning: Chi-squared approximation may be incorrect
```

Hide

```
print(chi_square_test)
```

```
    Pearson's Chi-squared test

data:  contingency_table
X-squared = 15.385, df = 3, p-value = 0.001516
```

Hide

```
fisher_exact_test <- fisher.test(contingency_table)
print(fisher_exact_test)
```

```
    Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.0003154
alternative hypothesis: two.sided
```
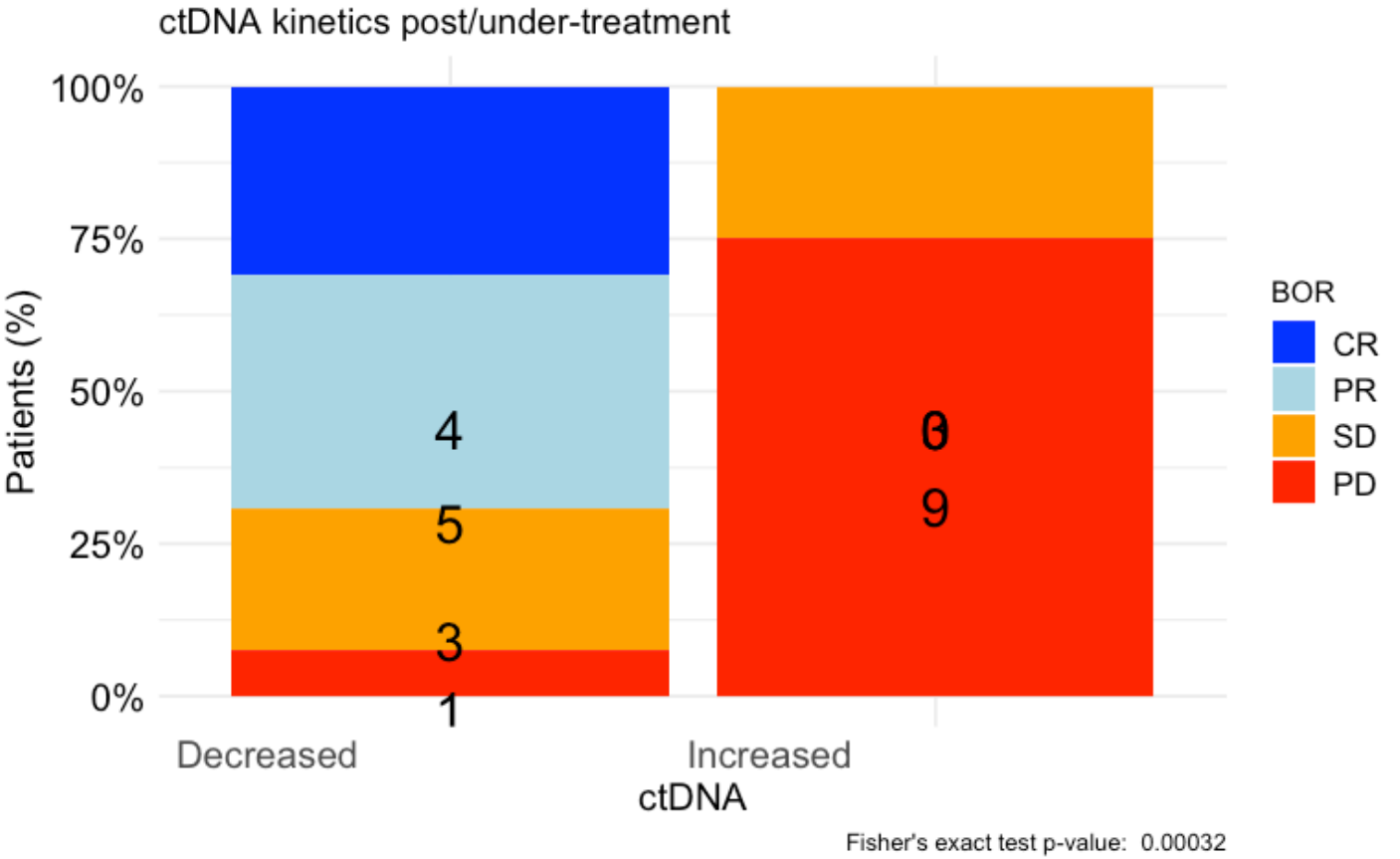
Hide

```
print(contingency_table)
```

```
            CR PR SD PD
  Decreased  4  5  3  1
  Increased  0  0  3  9
```

Hide

```r
table_df <- as.data.frame(contingency_table)
table_df$Total <- ave(table_df$Freq, table_df$Var1, FUN = sum)
table_df$Percentage <- table_df$Freq / table_df$Total
table_df$MiddlePercentage <- table_df$Percentage / 2
ggplot(table_df, aes(x = Var1, y = Percentage, fill = Var2)) +
  geom_bar(stat = "identity") +
  geom_text(aes(y = MiddlePercentage, label = Freq), position = "stack", color = "blac
k", vjust = 1.5, size = 7) +
  theme_minimal() +
  labs(title = "ctDNA kinetics post/under-treatment",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "BOR",
       caption = paste("Fisher's exact test p-value: ", format.pval(fisher_exact_test$p.
value))) +
  scale_y_continuous(labels = scales::percent_format()) +
  scale_fill_manual(values = c("CR" = "blue", "PR" = "lightblue", "SD" = "orange", "PD"
= "red")) + # define custom colors
  theme(axis.text.x = element_text(angle = 0, hjust = 1.5, size = 14), # increase x-axis
text size
        axis.text.y = element_text(size = 14, color = "black"), # increase y-axis text s
ize
        axis.title.x = element_text(size = 14, color = "black"), # increase x-axis label
size
        axis.title.y = element_text(size = 14, color = "black"), # increase y-axis label
size
        legend.text = element_text(size = 12, color = "black"))  # increase Progression
label size
```

ctDNA kinetics post/under-treatment

Fisher's exact test p-value: 0.00032

#PFS by ctDNA clearance post/during-ICI

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
circ_datadf <- as.data.frame(circ_data)

circ_data$ctDNA.Dynamics <- NA
circ_data <- circ_data %>%
  mutate(ctDNA.Dynamics = case_when(
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "NEGATIVE" ~ 1,
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "POSITIVE" ~ 2
  ))

survfit(Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)~ctDNA.Dynamics, d
ata = circ_data)
event_summary <- circ_data %>%
  group_by(ctDNA.Dynamics) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.Dynamics, data = circ_data,conf.int=0.95,conf.ty
pe="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRU
E, break.time.by=6, palette=c("blue","red"), title="PFS - ctDNA clearance Post/Under tre
atment", ylab= "Progression-Free Survival", xlab="Months from Start of Immunotherapy", l
egend.labs=c("Clearance", "No Clearance"), legend.title="")
summary(KM_curve, times= c(0, 12, 24, 36))
circ_data$ctDNA.Dynamics <- factor(circ_data$ctDNA.Dynamics, levels=c("1","2"), labels =
c("Clearance", "No Clearance"))
cox_fit <- coxph(surv_object ~ ctDNA.Dynamics, data=circ_data)
ggforest(cox_fit,data = circ_data)
summary(cox_fit)
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)

circ_data$PFS.Event <- factor(circ_data$PFS.Event, levels = c("FALSE", "TRUE"), labels =
c("No Progression", "Progression"))
```

```
contingency_table <- table(circ_data$ctDNA.Dynamics, circ_data$PFS.Event)
chi_square_test <- chisq.test(contingency_table)
print(chi_square_test)
fisher_exact_test <- fisher.test(contingency_table)
print(fisher_exact_test)
print(contingency_table)
table_df <- as.data.frame(contingency_table)
table_df$Total <- ave(table_df$Freq, table_df$Var1, FUN = sum)
table_df$Percentage <- table_df$Freq / table_df$Total
table_df$MiddlePercentage <- table_df$Percentage / 2
ggplot(table_df, aes(x = Var1, y = Percentage, fill = Var2)) +
  geom_bar(stat = "identity") +
  geom_text(aes(y = MiddlePercentage, label = Freq), position = "stack", color = "blac
k", vjust = 1.5, size = 7) +
  theme_minimal() +
  labs(title = "ctDNA clearance post/under-treatment",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "Progression",
       caption = paste("Fisher's exact test p-value: ", format.pval(fisher_exact_test$p.
value))) +
  scale_y_continuous(labels = scales::percent_format()) +
  scale_fill_manual(values = c("No Progression" = "blue", "Progression" = "red")) + # de
fine custom colors
  theme(axis.text.x = element_text(angle = 0, hjust = 1.5, size = 14), # increase x-axis
text size
        axis.text.y = element_text(size = 14, color = "black"), # increase y-axis text s
ize
        axis.title.x = element_text(size = 14, color = "black"), # increase x-axis label
size
        axis.title.y = element_text(size = 14, color = "black"), # increase y-axis label
size
        legend.text = element_text(size = 12, color = "black"))  # increase Progression
label size
```

#OS by ctDNA clearance post/during-ICI

Hide

```r
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
circ_datadf <- as.data.frame(circ_data)

circ_data$ctDNA.Dynamics <- NA
circ_data <- circ_data %>%
  mutate(ctDNA.Dynamics = case_when(
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "NEGATIVE" ~ 1,
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "POSITIVE" ~ 2
  ))

survfit(Surv(time = circ_data$OS.months, event = circ_data$OS.Event)~ctDNA.Dynamics, dat
a = circ_data)
```

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

                  n events median 0.95LCL 0.95UCL
ctDNA.Dynamics=1  6      2     NA   20.50      NA
ctDNA.Dynamics=2 19     16   9.82    7.75    26.7
```

Hide

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

ctDNA.Dynamics	Total	Events	Fraction	Percentage
<dbl>	<int>	<int>	<dbl>	<dbl>
1	6	2	0.3333333	33.33333
2	19	16	0.8421053	84.21053

2 rows

Hide

```
surv_object <-Surv(time = circ_data$OS.months, event = circ_data$OS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.Dynamics, data = circ_data,conf.int=0.95,conf.ty
pe="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRU
E, break.time.by=6, palette=c("blue","red"), title="OS - ctDNA clearance Post/Under trea
tment", ylab= "Overall Survival", xlab="Months from Start of Immunotherapy", legend.labs
=c("Clearance", "No Clearance"), legend.title="")
```

OS - ctDNA clearance Post/Under treatment
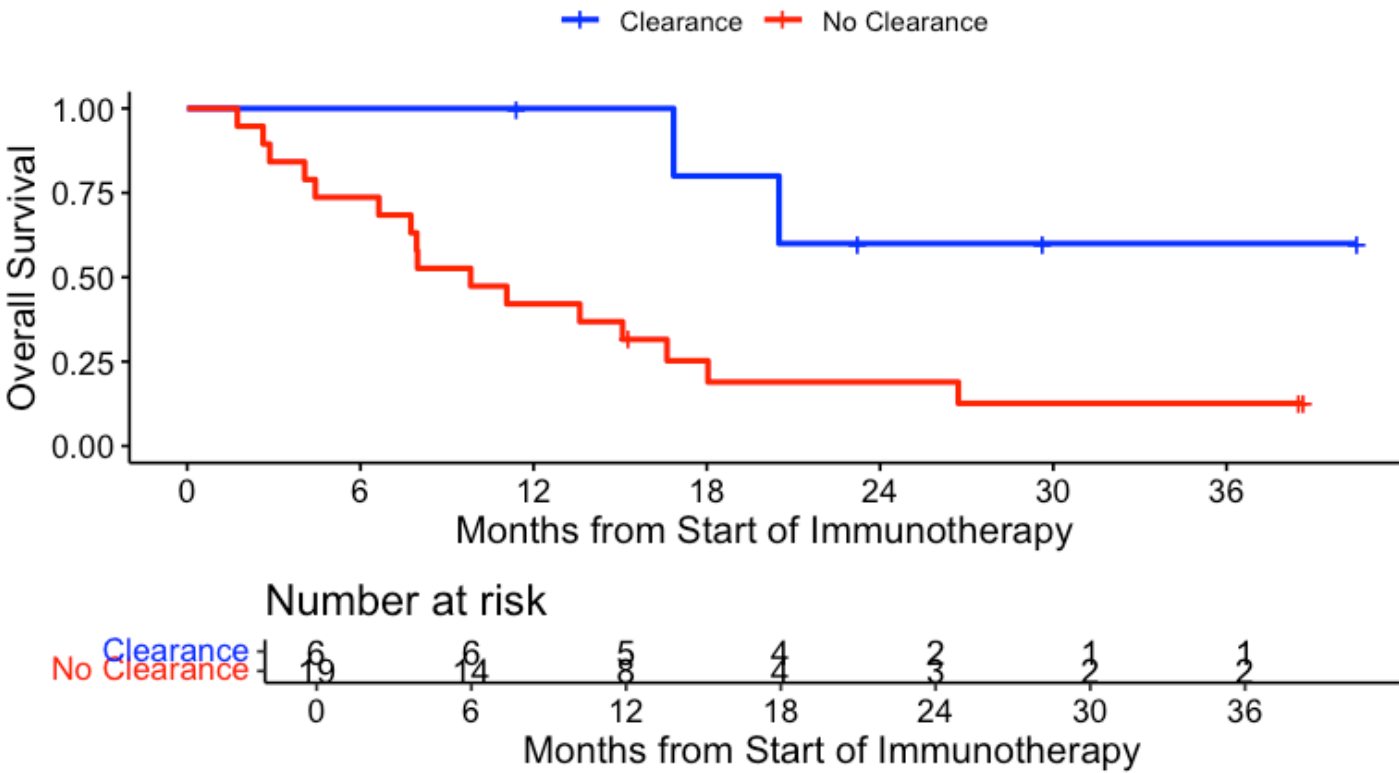

```
summary(KM_curve, times= c(0, 12, 24, 36))
```
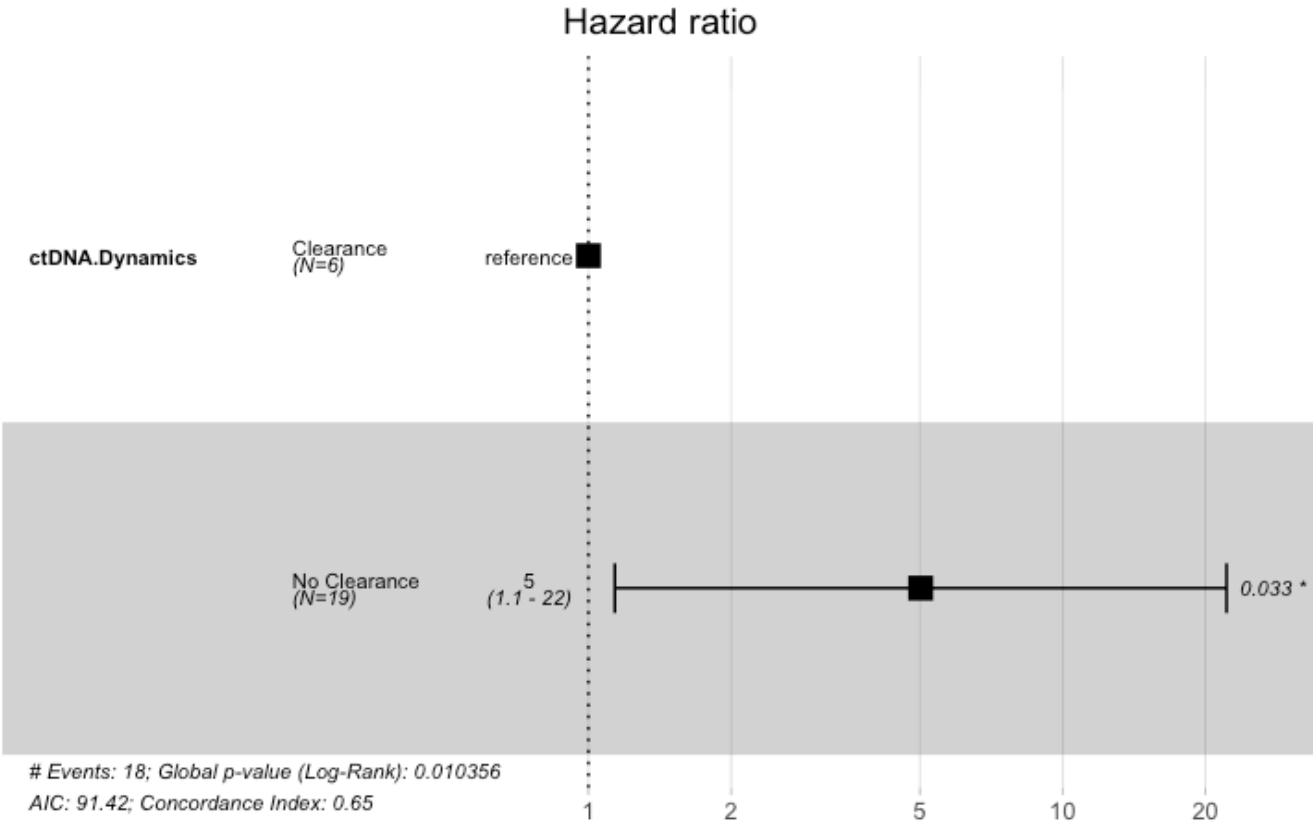
```
Call: survfit(formula = surv_object ~ ctDNA.Dynamics, data = circ_data,
    conf.int = 0.95, conf.type = "log-log")

                ctDNA.Dynamics=1
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0      6       0      1.0   0.000        1.000        1.000
   12      5       0      1.0   0.000           NA           NA
   24      2       2      0.6   0.219        0.126        0.882
   36      1       0      0.6   0.219        0.126        0.882

                ctDNA.Dynamics=2
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0     19       0    1.000  0.0000       1.0000        1.000
   12      8      11    0.421  0.1133       0.2037        0.625
   24      3       4    0.189  0.0942       0.0503        0.396
   36      2       1    0.126  0.0813       0.0222        0.325
```

Hide

```
circ_data$ctDNA.Dynamics <- factor(circ_data$ctDNA.Dynamics, levels=c("1","2"), labels =
c("Clearance", "No Clearance"))
cox_fit <- coxph(surv_object ~ ctDNA.Dynamics, data=circ_data)
ggforest(cox_fit,data = circ_data)
```

Hazard ratio

| ctDNA.Dynamics | Clearance (N=6) | reference |
| No Clearance (N=19) | 5 (1.1 - 22) | 0.033 * |

Events: 18; Global p-value (Log-Rank): 0.010356
AIC: 91.42; Concordance Index: 0.65

Hide

```
summary(cox_fit)
```

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

  n= 25, number of events= 18

                             coef exp(coef) se(coef)      z Pr(>|z|)
ctDNA.DynamicsNo Clearance 1.6133    5.0196   0.7578 2.129   0.0333 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                           exp(coef) exp(-coef) lower .95 upper .95
ctDNA.DynamicsNo Clearance      5.02     0.1992     1.137     22.17

Concordance= 0.65  (se = 0.047 )
Likelihood ratio test= 6.57  on 1 df,    p=0.01
Wald test            = 4.53  on 1 df,    p=0.03
Score (logrank) test = 5.52  on 1 df,    p=0.02
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 5.02 (1.14-22.17); p = 0.033"
```

Hide

```
circ_data$OS.Event <- factor(circ_data$OS.Event, levels = c("FALSE", "TRUE"), labels = c
("Alive", "Deceased"))
contingency_table <- table(circ_data$ctDNA.Dynamics, circ_data$OS.Event)
chi_square_test <- chisq.test(contingency_table)
```

```
Warning: Chi-squared approximation may be incorrect
```

Hide

```
print(chi_square_test)
```

```
	Pearson's Chi-squared test with Yates' continuity correction

data:  contingency_table
X-squared = 3.6032, df = 1, p-value = 0.05767
```

Hide

```
fisher_exact_test <- fisher.test(contingency_table)
print(fisher_exact_test)
```

```
	Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.03241
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
   0.8988842 150.9697807
sample estimates:
odds ratio
   9.34674
```
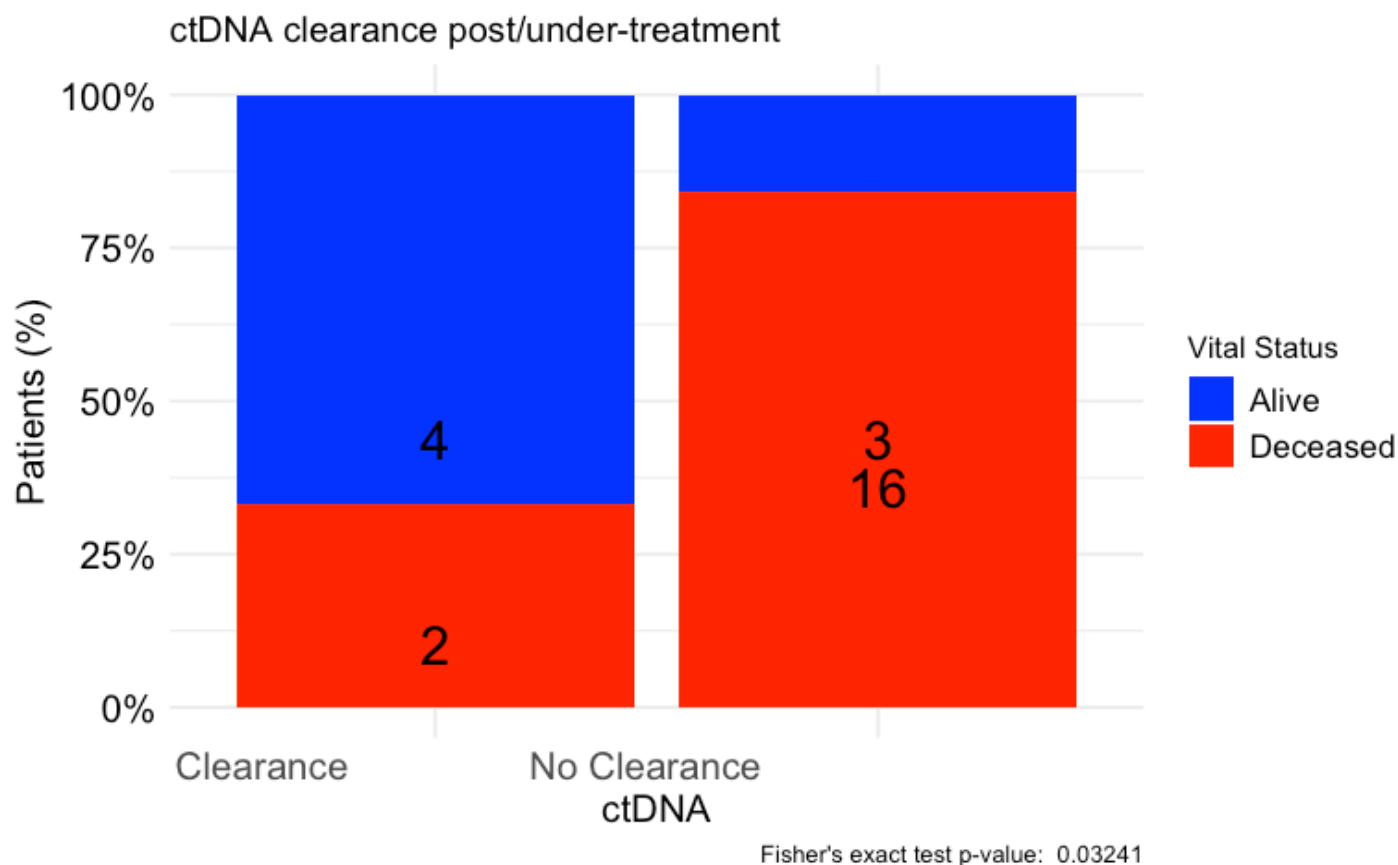
Hide

```
print(contingency_table)
```

```
              Alive Deceased
  Clearance       4        2
  No Clearance    3       16
```

Hide

```
table_df <- as.data.frame(contingency_table)
table_df$Total <- ave(table_df$Freq, table_df$Var1, FUN = sum)
table_df$Percentage <- table_df$Freq / table_df$Total
table_df$MiddlePercentage <- table_df$Percentage / 2
ggplot(table_df, aes(x = Var1, y = Percentage, fill = Var2)) +
  geom_bar(stat = "identity") +
  geom_text(aes(y = MiddlePercentage, label = Freq), position = "stack", color = "blac
k", vjust = 1.5, size = 7) +
  theme_minimal() +
  labs(title = "ctDNA clearance post/under-treatment",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "Vital Status",
       caption = paste("Fisher's exact test p-value: ", format.pval(fisher_exact_test$p.
value))) +
  scale_y_continuous(labels = scales::percent_format()) +
  scale_fill_manual(values = c("Alive" = "blue", "Deceased" = "red")) + # define custom
colors
  theme(axis.text.x = element_text(angle = 0, hjust = 1.5, size = 14), # increase x-axis
text size
        axis.text.y = element_text(size = 14, color = "black"), # increase y-axis text s
ize
        axis.title.x = element_text(size = 14, color = "black"), # increase x-axis label
size
        axis.title.y = element_text(size = 14, color = "black"), # increase y-axis label
size
        legend.text = element_text(size = 12, color = "black"))  # increase Progression
label size
```

ctDNA clearance post/under-treatment

Fisher's exact test p-value: 0.03241

#Association of ctDNA clearance post/during-ICI with BOR

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
circ_datadf <- as.data.frame(circ_data)

circ_data$ctDNA.Dynamics <- NA
circ_data <- circ_data %>%
  mutate(ctDNA.Dynamics = case_when(
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "NEGATIVE" ~ 1,
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "POSITIVE" ~ 2
  ))

circ_data$ctDNA.Dynamics <- factor(circ_data$ctDNA.Dynamics, levels=c("1","2"), labels =
c("Clearance", "No Clearance"))
circ_data$RESIST <- factor(circ_data$RESIST, levels = c("CR", "PR", "SD", "PD"))
contingency_table <- table(circ_data$ctDNA.Dynamics, circ_data$RESIST)
chi_square_test <- chisq.test(contingency_table)
```

```
Warning: Chi-squared approximation may be incorrect
```

```
print(chi_square_test)
```

```
	Pearson's Chi-squared test

data:  contingency_table
X-squared = 14.309, df = 3, p-value = 0.002513
```

```
fisher_exact_test <- fisher.test(contingency_table)
print(fisher_exact_test)
```

```
	Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.001129
alternative hypothesis: two.sided
```

```
print(contingency_table)
```

```
              CR PR SD PD
  Clearance    3  3  0  0
  No Clearance 1  2  6 10
```
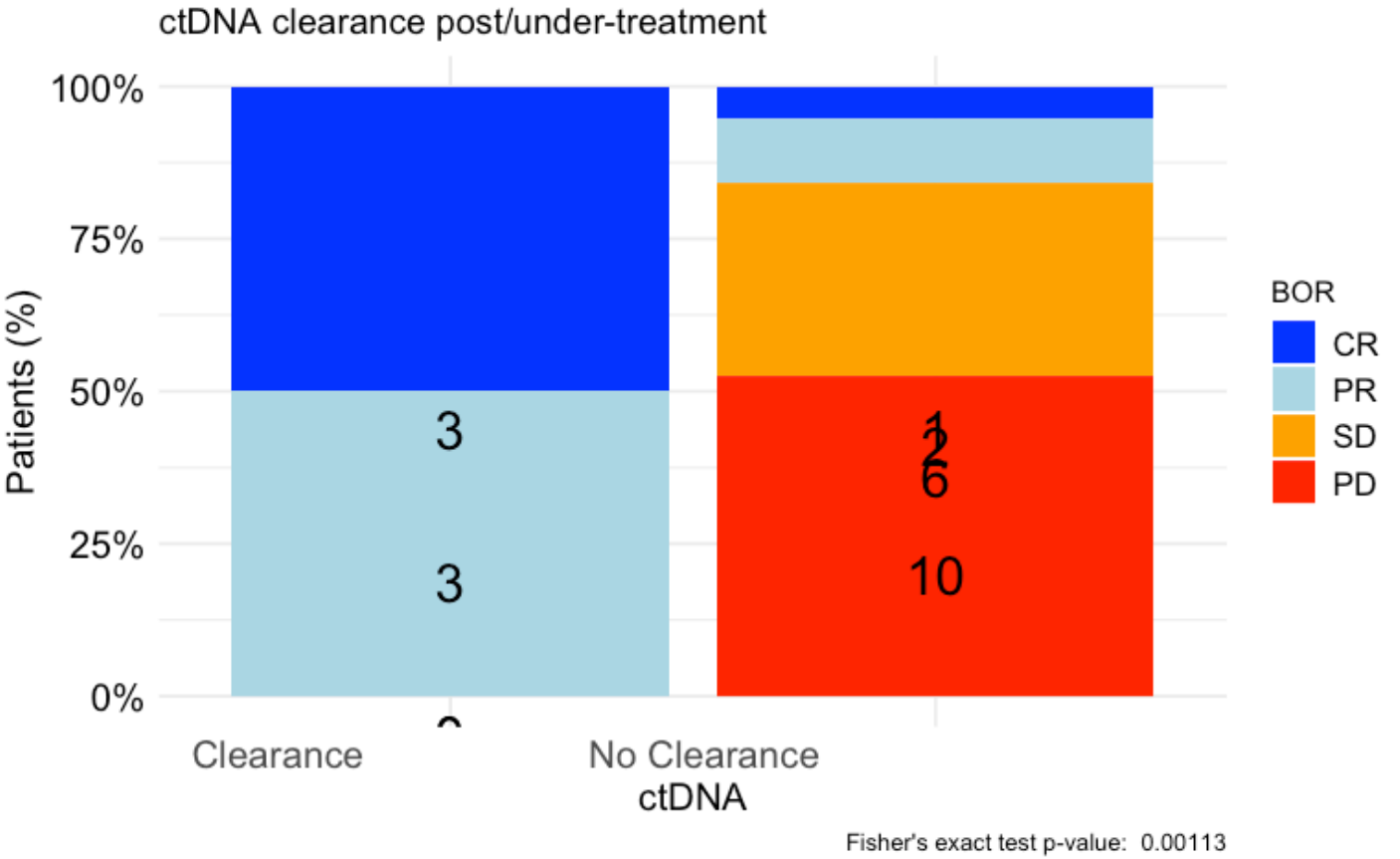
```
table_df <- as.data.frame(contingency_table)
table_df$Total <- ave(table_df$Freq, table_df$Var1, FUN = sum)
table_df$Percentage <- table_df$Freq / table_df$Total
table_df$MiddlePercentage <- table_df$Percentage / 2
ggplot(table_df, aes(x = Var1, y = Percentage, fill = Var2)) +
  geom_bar(stat = "identity") +
  geom_text(aes(y = MiddlePercentage, label = Freq), position = "stack", color = "blac
k", vjust = 1.5, size = 7) +
  theme_minimal() +
  labs(title = "ctDNA clearance post/under-treatment",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "BOR",
       caption = paste("Fisher's exact test p-value: ", format.pval(fisher_exact_test$p.
value))) +
  scale_y_continuous(labels = scales::percent_format()) +
  scale_fill_manual(values = c("CR" = "blue", "PR" = "lightblue", "SD" = "orange", "PD"
= "red")) + # define custom colors
  theme(axis.text.x = element_text(angle = 0, hjust = 1.5, size = 14), # increase x-axis
text size
        axis.text.y = element_text(size = 14, color = "black"), # increase y-axis text s
ize
        axis.title.x = element_text(size = 14, color = "black"), # increase x-axis label
size
        axis.title.y = element_text(size = 14, color = "black"), # increase y-axis label
size
        legend.text = element_text(size = 12, color = "black"))  # increase Progression
label size
```

ctDNA clearance post/under-treatment

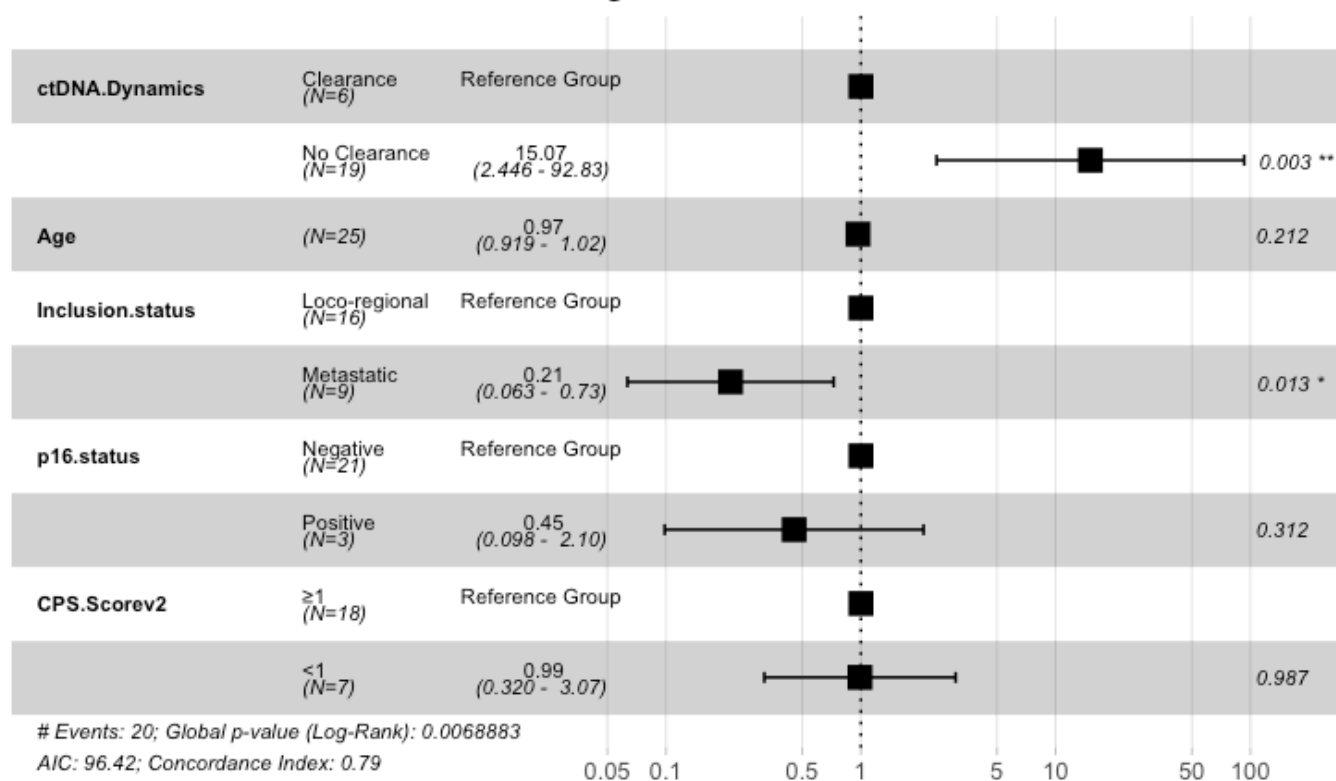#Multivariate cox regression for PFS

Hide

```r
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
circ_datadf <- as.data.frame(circ_data)

circ_data$ctDNA.Dynamics <- NA
circ_data <- circ_data %>%
  mutate(ctDNA.Dynamics = case_when(
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "NEGATIVE" ~ 1,
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "POSITIVE" ~ 2
  ))

circ_data$ctDNA.Dynamics <- factor(circ_data$ctDNA.Dynamics, levels=c("1","2"), labels =
c("Clearance", "No Clearance"))
circ_data$Inclusion.status <- factor(circ_data$Inclusion.status, levels = c("Loco-region
al", "Metastatic"))
circ_data$p16.status <- factor(circ_data$p16.status, levels = c("Negative", "Positive"))
circ_data$CPS.Scorev2 <- factor(circ_data$CPS.Scorev2, levels = c("≥1", "<1"))
surv_object <- Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
cox_fit <- coxph(surv_object ~ ctDNA.Dynamics + Age + Inclusion.status + p16.status + CP
S.Scorev2, data=circ_data)
ggforest(cox_fit, data = circ_data, main = "Multivariate Regression Model for PFS", refL
abel = "Reference Group")
```

Multivariate Regression Model for PFS

ctDNA.Dynamics	Clearance (N=6)	Reference Group	■		
	No Clearance (N=19)	15.07 (2.446 - 92.83)	├──■──┤		0.003 **
Age	(N=25)	0.97 (0.919 - 1.02)	■		0.212
Inclusion.status	Loco-regional (N=16)	Reference Group	■		
	Metastatic (N=9)	0.21 (0.063 - 0.73)	├──■──┤		0.013 *
p16.status	Negative (N=21)	Reference Group	■		
	Positive (N=3)	0.45 (0.098 - 2.10)	├──■──┤		0.312
CPS.Scorev2	≥1 (N=18)	Reference Group	■		
	<1 (N=7)	0.99 (0.320 - 3.07)	├──■──┤		0.987

 # Events: 20; Global p-value (Log-Rank): 0.0068883
AIC: 96.42; Concordance Index: 0.79

```
0.05  0.1      0.5   1      5   10      50  100
```

Hide

```
test.ph <- cox.zph(cox_fit)
```

#Univariate PFS cox regression for variables included in MVA

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]

circ_data$ctDNA.Dynamics <- NA
circ_data <- circ_data %>%
  mutate(ctDNA.Dynamics = case_when(
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "NEGATIVE" ~ 1,
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "POSITIVE" ~ 2
  ))
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
circ_data$ctDNA.Dynamics <- factor(circ_data$ctDNA.Dynamics, levels=c("1","2"), labels =
c("Clearance", "No Clearance")) #univariate for ctDNA clearance post-treatment
cox_fit <- coxph(surv_object ~ ctDNA.Dynamics, data=circ_data)
summary(cox_fit)
```

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

  n= 25, number of events= 20

                              coef exp(coef) se(coef)      z Pr(>|z|)
ctDNA.DynamicsNo Clearance 2.085     8.048    0.767 2.719  0.00655 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                           exp(coef) exp(-coef) lower .95 upper .95
ctDNA.DynamicsNo Clearance     8.048     0.1243      1.79     36.19

Concordance= 0.672  (se = 0.049 )
Likelihood ratio test= 11.61  on 1 df,    p=7e-04
Wald test            = 7.39  on 1 df,    p=0.007
Score (logrank) test = 9.78  on 1 df,    p=0.002
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 8.05 (1.79-36.19); p = 0.007"
```

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
cox_fit <- coxph(surv_object ~ Age, data=circ_data) #univariate for age
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ Age, data = circ_data)

  n= 25, number of events= 20

        coef exp(coef) se(coef)      z Pr(>|z|)
Age -0.02566   0.97467  0.02392 -1.073    0.283

    exp(coef) exp(-coef) lower .95 upper .95
Age    0.9747      1.026      0.93     1.021


Concordance= 0.512  (se = 0.079 )
Likelihood ratio test= 1.12  on 1 df,    p=0.3
Wald test            = 1.15  on 1 df,    p=0.3
Score (logrank) test = 1.16  on 1 df,    p=0.3
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 0.97 (0.93-1.02); p = 0.283"
```

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
circ_data$Inclusion.status <- factor(circ_data$Inclusion.status, levels = c("Loco-region
al", "Metastatic")) #univariate for Stage/disease status
cox_fit <- coxph(surv_object ~ Inclusion.status, data=circ_data)
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ Inclusion.status, data = circ_data)

  n= 25, number of events= 20

                            coef exp(coef) se(coef)      z Pr(>|z|)
Inclusion.statusMetastatic -0.1110    0.8949   0.4582 -0.242    0.809

                          exp(coef) exp(-coef) lower .95 upper .95
Inclusion.statusMetastatic    0.8949      1.117    0.3646     2.197

Concordance= 0.556  (se = 0.056 )
Likelihood ratio test= 0.06  on 1 df,    p=0.8
Wald test            = 0.06  on 1 df,    p=0.8
Score (logrank) test = 0.06  on 1 df,    p=0.8
```

Hide

```r
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 0.89 (0.36-2.2); p = 0.809"
```

Hide

```r
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
circ_data$p16.status <- factor(circ_data$p16.status, levels = c("Negative", "Positive"))
#univariate for p16 status
cox_fit <- coxph(surv_object ~ p16.status, data=circ_data)
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ p16.status, data = circ_data)

  n= 24, number of events= 20
   (1 observation deleted due to missingness)

                     coef exp(coef) se(coef)    z Pr(>|z|)
p16.statusPositive 0.2431    1.2752   0.6402 0.38    0.704

                  exp(coef) exp(-coef) lower .95 upper .95
p16.statusPositive    1.275     0.7842    0.3636     4.472

Concordance= 0.491  (se = 0.027 )
Likelihood ratio test= 0.14  on 1 df,    p=0.7
Wald test            = 0.14  on 1 df,    p=0.7
Score (logrank) test = 0.14  on 1 df,    p=0.7
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 1.28 (0.36-4.47); p = 0.704"
```

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
surv_object <-Surv(time = circ_data$PFS.months, event = circ_data$PFS.Event)
circ_data$CPS.Scorev2 <- factor(circ_data$CPS.Scorev2, levels = c("≥1", "<1")) #univaria
te for CPS score
cox_fit <- coxph(surv_object ~ CPS.Scorev2, data=circ_data)
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ CPS.Scorev2, data = circ_data)

  n= 25, number of events= 20

                coef exp(coef) se(coef)      z Pr(>|z|)
CPS.Scorev2<1 0.3866    1.4720   0.4905 0.788    0.431

              exp(coef) exp(-coef) lower .95 upper .95
CPS.Scorev2<1     1.472     0.6793    0.5628      3.85


Concordance= 0.564  (se = 0.062 )
Likelihood ratio test= 0.59  on 1 df,    p=0.4
Wald test            = 0.62  on 1 df,    p=0.4
Score (logrank) test = 0.63  on 1 df,    p=0.4
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 1.47 (0.56-3.85); p = 0.431"
```

#Univariate OS cox regression for variables included in MVA

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]

circ_data$ctDNA.Dynamics <- NA
circ_data <- circ_data %>%
  mutate(ctDNA.Dynamics = case_when(
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "NEGATIVE" ~ 1,
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "POSITIVE" ~ 2
  ))
surv_object <-Surv(time = circ_data$OS.months, event = circ_data$OS.Event)
circ_data$ctDNA.Dynamics <- factor(circ_data$ctDNA.Dynamics, levels=c("1","2"), labels =
c("Clearance", "No Clearance")) #univariate for ctDNA clearance post-treatment
cox_fit <- coxph(surv_object ~ ctDNA.Dynamics, data=circ_data)
summary(cox_fit)
```

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

  n= 25, number of events= 18

                              coef exp(coef) se(coef)      z Pr(>|z|)
ctDNA.DynamicsNo Clearance 1.6133    5.0196   0.7578 2.129   0.0333 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

                           exp(coef) exp(-coef) lower .95 upper .95
ctDNA.DynamicsNo Clearance      5.02     0.1992     1.137     22.17

Concordance= 0.65  (se = 0.047 )
Likelihood ratio test= 6.57  on 1 df,    p=0.01
Wald test            = 4.53  on 1 df,    p=0.03
Score (logrank) test = 5.52  on 1 df,    p=0.02
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 5.02 (1.14-22.17); p = 0.033"
```

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
surv_object <-Surv(time = circ_data$OS.months, event = circ_data$OS.Event)
cox_fit <- coxph(surv_object ~ Age, data=circ_data) #univariate for age
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ Age, data = circ_data)

  n= 25, number of events= 18

        coef exp(coef) se(coef)      z Pr(>|z|)
Age -0.03681   0.96386  0.02368 -1.554     0.12

    exp(coef) exp(-coef) lower .95 upper .95
Age    0.9639      1.037    0.9201      1.01

Concordance= 0.568  (se = 0.073 )
Likelihood ratio test= 2.34  on 1 df,    p=0.1
Wald test            = 2.42  on 1 df,    p=0.1
Score (logrank) test = 2.46  on 1 df,    p=0.1
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 0.96 (0.92-1.01); p = 0.12"
```

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
surv_object <-Surv(time = circ_data$OS.months, event = circ_data$OS.Event)
circ_data$Inclusion.status <- factor(circ_data$Inclusion.status, levels = c("Loco-region
al", "Metastatic")) #univariate for Stage/disease status
cox_fit <- coxph(surv_object ~ Inclusion.status, data=circ_data)
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ Inclusion.status, data = circ_data)

  n= 25, number of events= 18

                                coef exp(coef) se(coef)      z Pr(>|z|)
Inclusion.statusMetastatic   -0.2314    0.7934   0.4852 -0.477    0.633

                          exp(coef) exp(-coef) lower .95 upper .95
Inclusion.statusMetastatic    0.7934       1.26    0.3065     2.054

Concordance= 0.583  (se = 0.055 )
Likelihood ratio test= 0.23  on 1 df,    p=0.6
Wald test            = 0.23  on 1 df,    p=0.6
Score (logrank) test = 0.23  on 1 df,    p=0.6
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 0.79 (0.31-2.05); p = 0.633"
```

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
surv_object <-Surv(time = circ_data$OS.months, event = circ_data$OS.Event)
circ_data$p16.status <- factor(circ_data$p16.status, levels = c("Negative", "Positive"))
#univariate for p16 status
cox_fit <- coxph(surv_object ~ p16.status, data=circ_data)
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ p16.status, data = circ_data)

  n= 24, number of events= 18
   (1 observation deleted due to missingness)

                    coef exp(coef) se(coef)      z Pr(>|z|)
p16.statusPositive 0.1126    1.1192   0.6349 0.177    0.859

                exp(coef) exp(-coef) lower .95 upper .95
p16.statusPositive    1.119     0.8935    0.3224     3.885


Concordance= 0.486  (se = 0.034 )
Likelihood ratio test= 0.03  on 1 df,    p=0.9
Wald test           = 0.03  on 1 df,    p=0.9
Score (logrank) test = 0.03  on 1 df,    p=0.9
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 1.12 (0.32-3.88); p = 0.859"
```

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
surv_object <-Surv(time = circ_data$OS.months, event = circ_data$OS.Event)
circ_data$CPS.Scorev2 <- factor(circ_data$CPS.Scorev2, levels = c("≥1", "<1")) #univaria
te for CPS score
cox_fit <- coxph(surv_object ~ CPS.Scorev2, data=circ_data)
summary(cox_fit)
```

```
Call:
coxph(formula = surv_object ~ CPS.Scorev2, data = circ_data)

  n= 25, number of events= 18

                coef exp(coef) se(coef)      z Pr(>|z|)
CPS.Scorev2<1 0.4499    1.5682   0.5023 0.896     0.37

            exp(coef) exp(-coef) lower .95 upper .95
CPS.Scorev2<1     1.568     0.6377     0.586     4.197

Concordance= 0.555  (se = 0.061 )
Likelihood ratio test= 0.76  on 1 df,    p=0.4
Wald test            = 0.8  on 1 df,    p=0.4
Score (logrank) test = 0.82  on 1 df,    p=0.4
```

Hide

```
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_C
I, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 1.57 (0.59-4.2); p = 0.37"
```

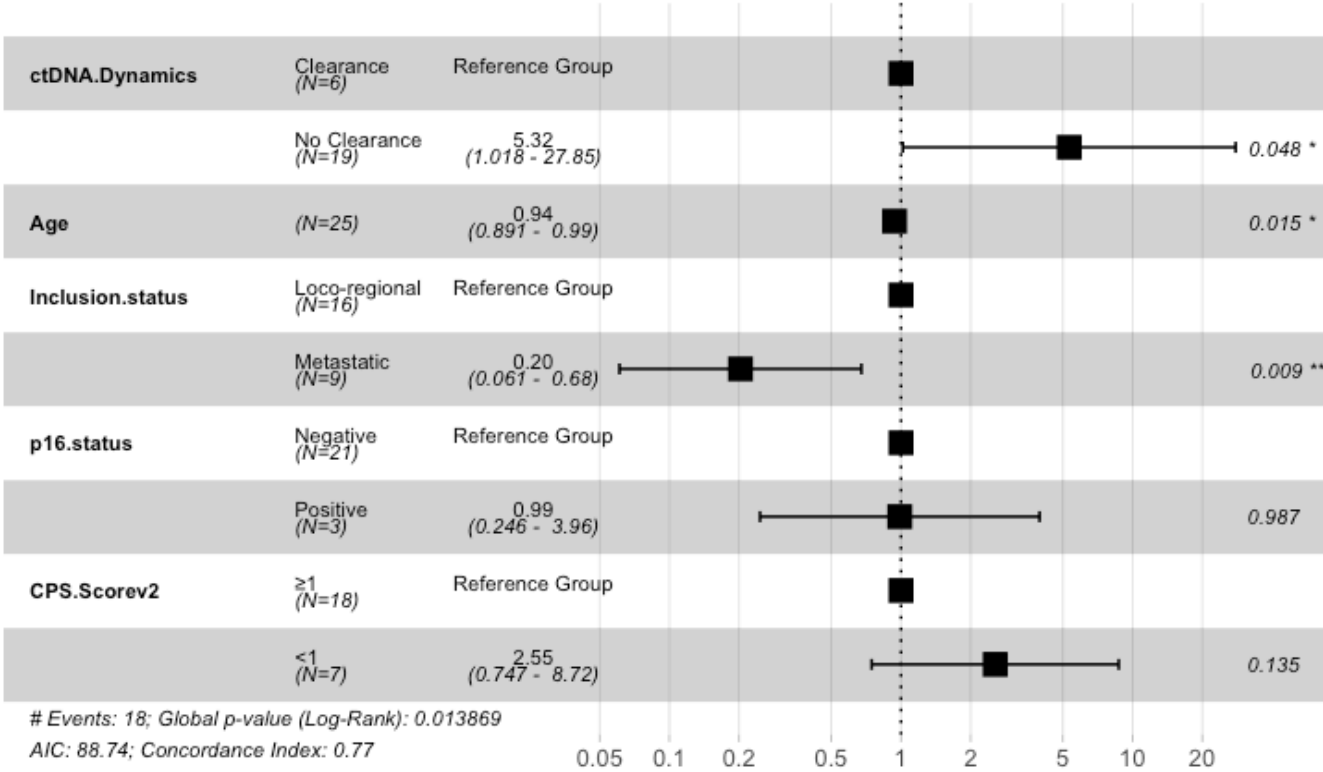#Multivariate cox regression for OS

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
circ_datadf <- as.data.frame(circ_data)

circ_data$ctDNA.Dynamics <- NA
circ_data <- circ_data %>%
  mutate(ctDNA.Dynamics = case_when(
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "NEGATIVE" ~ 1,
    ctDNA.Base == "POSITIVE" & ctDNA.postTx == "POSITIVE" ~ 2
  ))

circ_data$ctDNA.Dynamics <- factor(circ_data$ctDNA.Dynamics, levels=c("1","2"), labels =
c("Clearance", "No Clearance"))
circ_data$Inclusion.status <- factor(circ_data$Inclusion.status, levels = c("Loco-region
al", "Metastatic"))
circ_data$p16.status <- factor(circ_data$p16.status, levels = c("Negative", "Positive"))
circ_data$CPS.Scorev2 <- factor(circ_data$CPS.Scorev2, levels = c("≥1", "<1"))
surv_object <- Surv(time = circ_data$OS.months, event = circ_data$OS.Event)
cox_fit <- coxph(surv_object ~ ctDNA.Dynamics + Age + Inclusion.status + p16.status + CP
S.Scorev2, data=circ_data)
ggforest(cox_fit, data = circ_data, main = "Multivariate Regression Model for OS", refLa
bel = "Reference Group")
```

Multivariate Regression Model for OS

ctDNA.Dynamics	Clearance (N=6)	Reference Group		
	No Clearance (N=19)	5.32 (1.018 - 27.85)		0.048 *
Age	(N=25)	0.94 (0.891 - 0.99)		0.015 *
Inclusion.status	Loco-regional (N=16)	Reference Group		
	Metastatic (N=9)	0.20 (0.061 - 0.68)		0.009 **
p16.status	Negative (N=21)	Reference Group		
	Positive (N=3)	0.99 (0.246 - 3.96)		0.987
CPS.Scorev2	≥1 (N=18)	Reference Group		
	<1 (N=7)	2.55 (0.747 - 8.72)		0.135

Events: 18; Global p-value (Log-Rank): 0.013869
AIC: 88.74; Concordance Index: 0.77

0.05 0.1 0.2 0.5 1 2 5 10 20

Hide

```
test.ph <- cox.zph(cox_fit)
```