

EORTC HNSCC ICI Honore et al_02032025

Code ▾

```
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$ctDNA.Base == "POSITIVE", by=list(circ_data$Stage), FUN=sum)
total_counts_by_stage <- aggregate(circ_data$ctDNA.Base, by=list(circ_data$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.Base == "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 <fctr>	Total_Count <int>	Positive_Count <int>	Rate <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","POSITIVE"))
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$x) * 100 # Convert to percentage
)
combined_data$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 # 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 <fctr>	Total_Count <int>	Positive_Count <int>	Rate <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

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```

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")

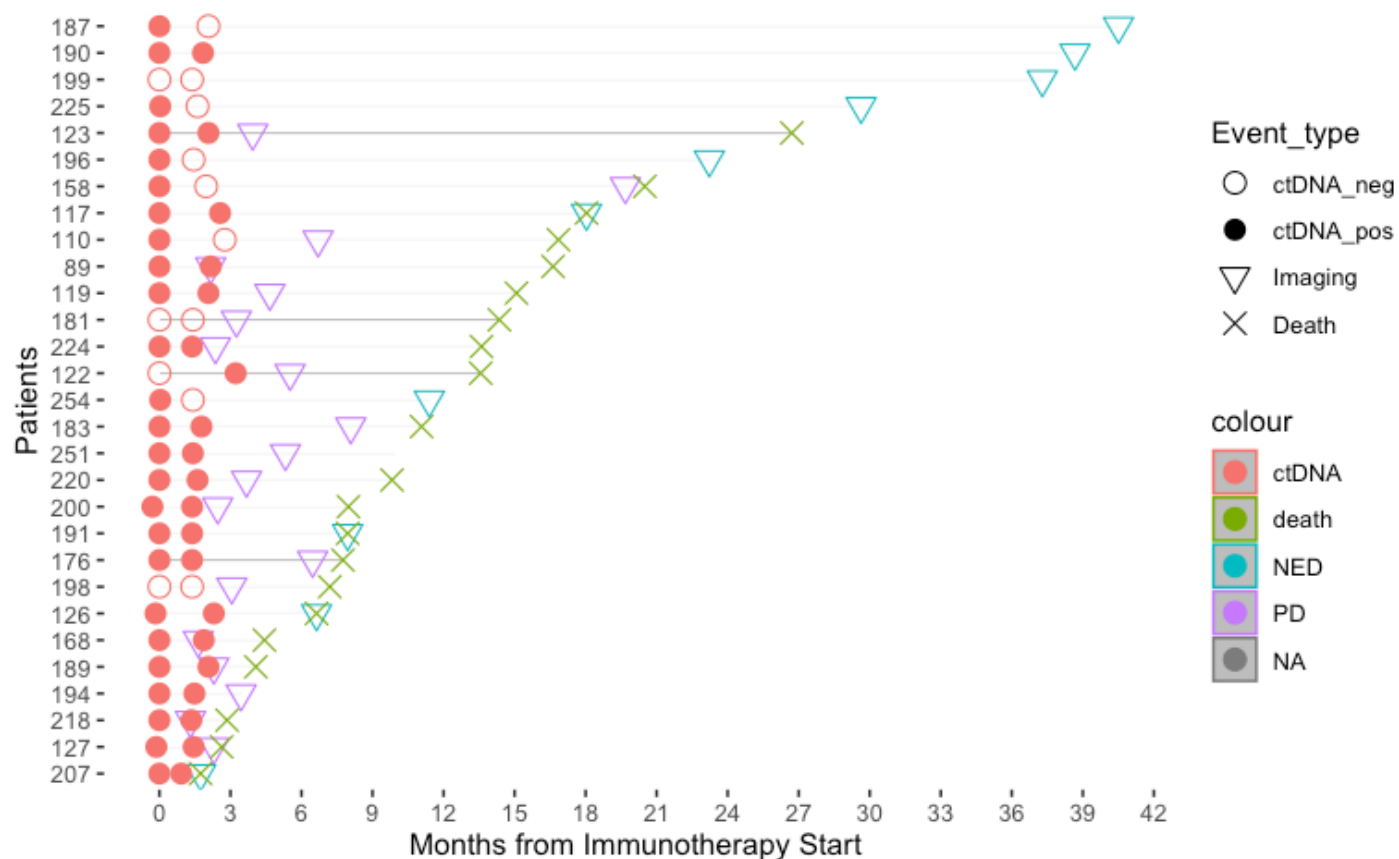
# Adding swimmer points
oplot_ev1 <- oplot + swimmer_points(df_points=clinstage_df,
                                   id='PatientName',
                                   time='date.diff.months',
                                   name_shape = 'Event_type',
                                   name_col = '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_pos',
                                                         'Imaging','Death'))

# Display the plot
oplot_ev1.1

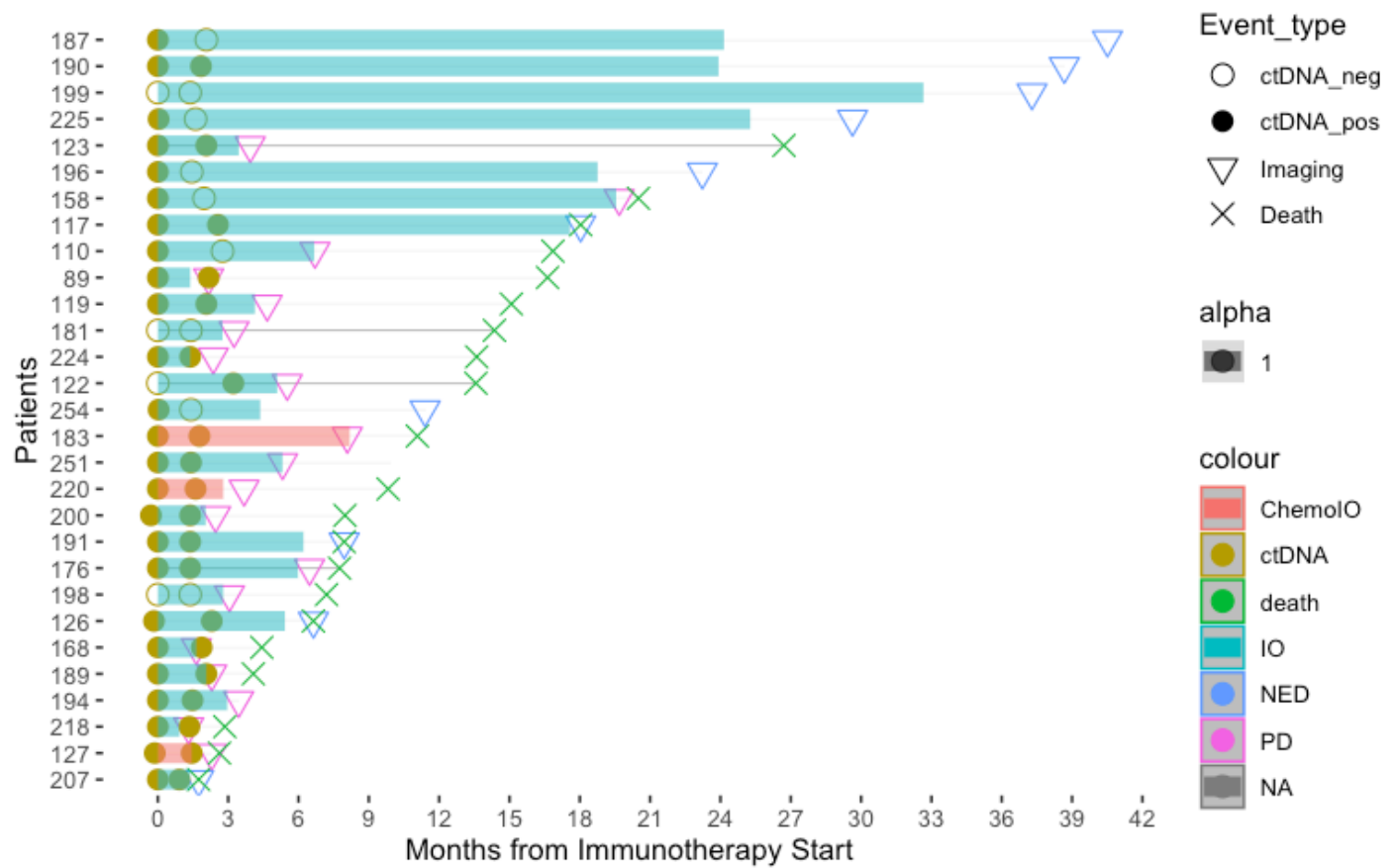
```


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```

oplot_ev2 <- oplot_ev1.1 + swimmer_lines(df_lines=c(
  instage_df,
  Name',
  tart.months',
  d.months',
  x_type',
  = 1.0)
  id='Patient
  start='Tx_s
  end='Tx_en
  name_col='T
  size=3.5,
  name_alpha
  oplot_ev2 <- oplot_ev2 + guides(linetype = guide_leg
end(override.aes = list(size = 5, color = "black")))
oplot_ev2

```

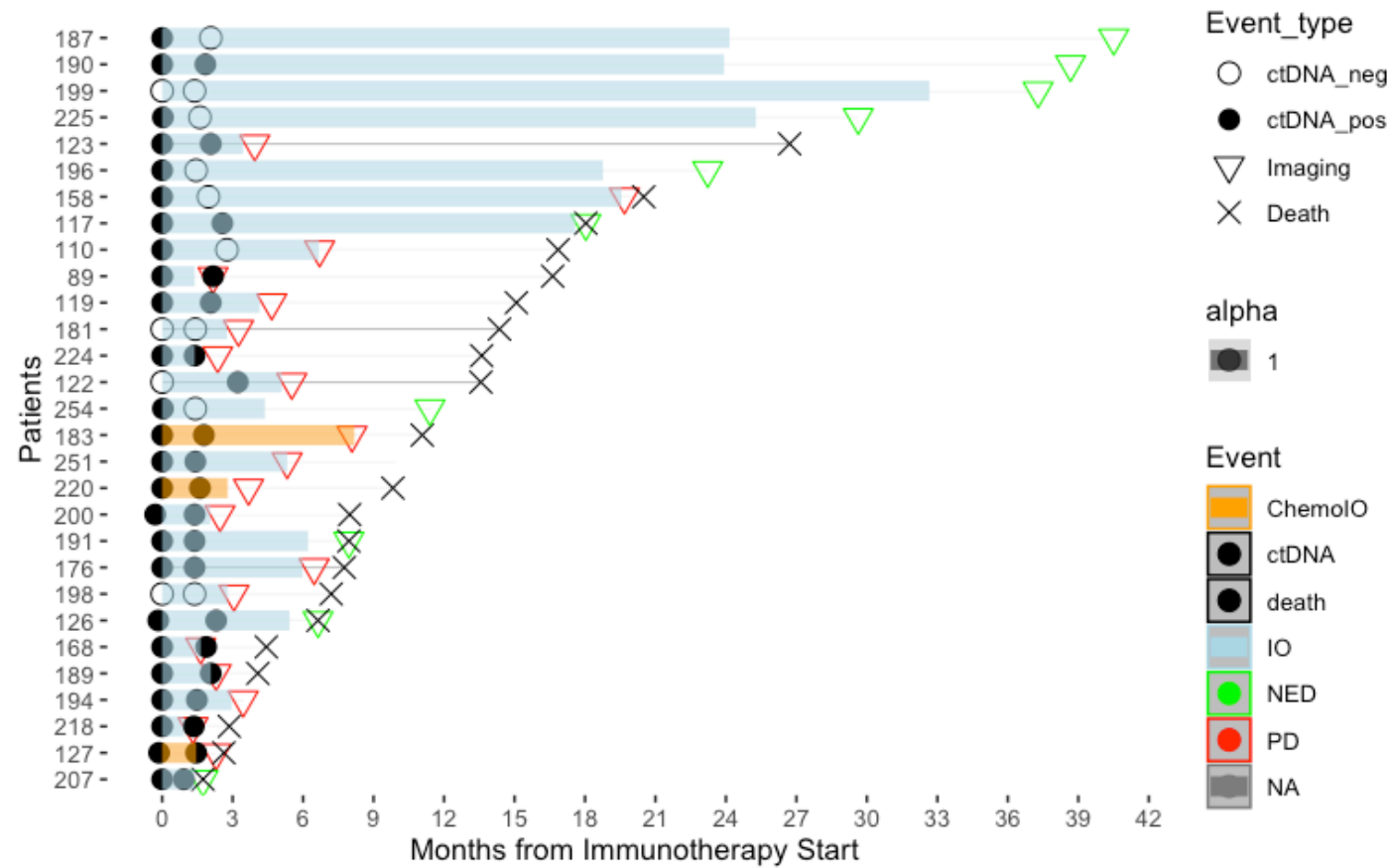


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```

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

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```

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 Start")

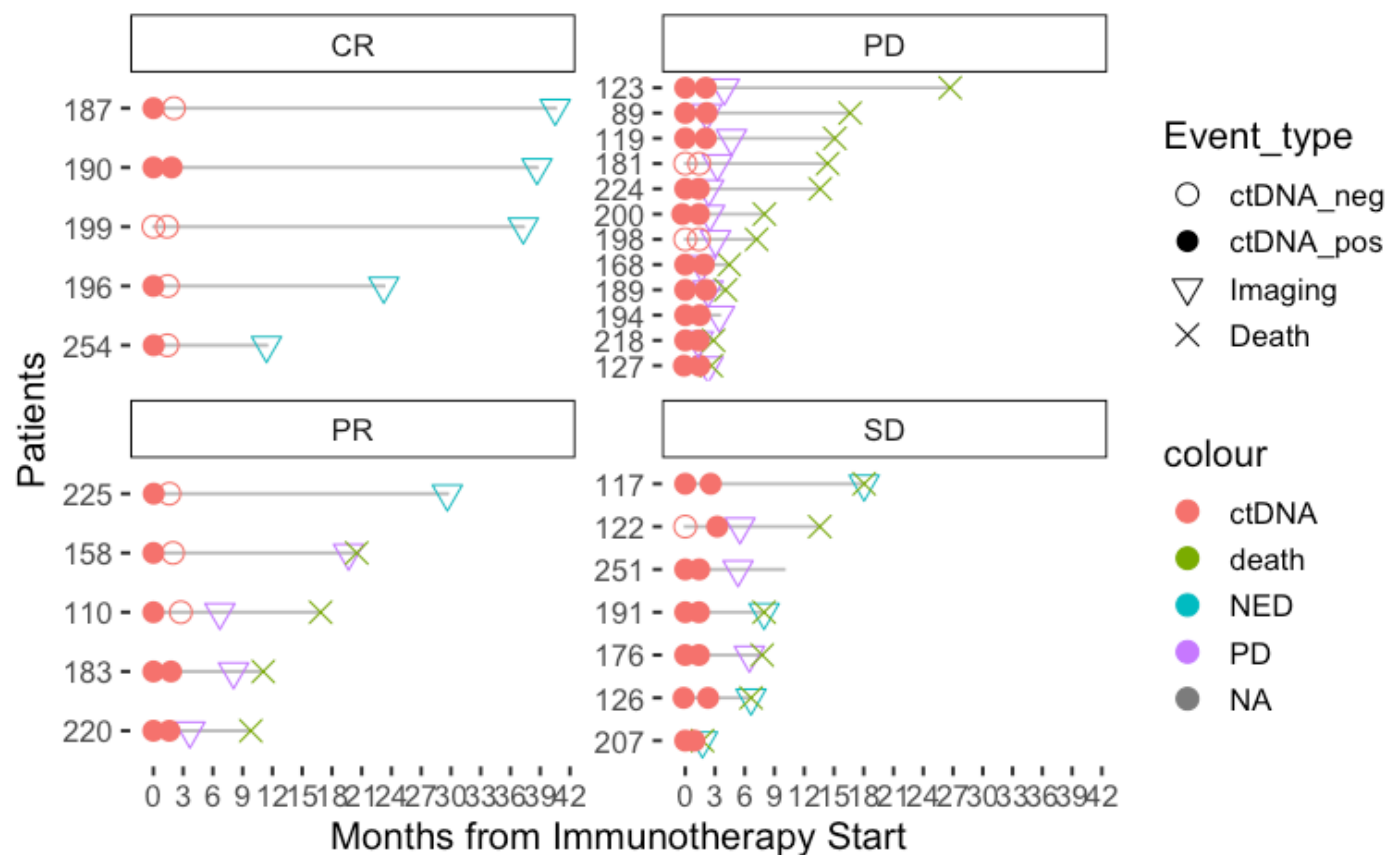
# Adding swimmer points
oplot_ev1 <- oplot_stratify + swimmer_points(df_points=clinstage_df,
                                             id='PatientName',
                                             time='date.diff.months',
                                             name_shape = 'Event_type',
                                             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_pos','Imaging','Death'))

# Display the plot
oplot_ev1.1

```

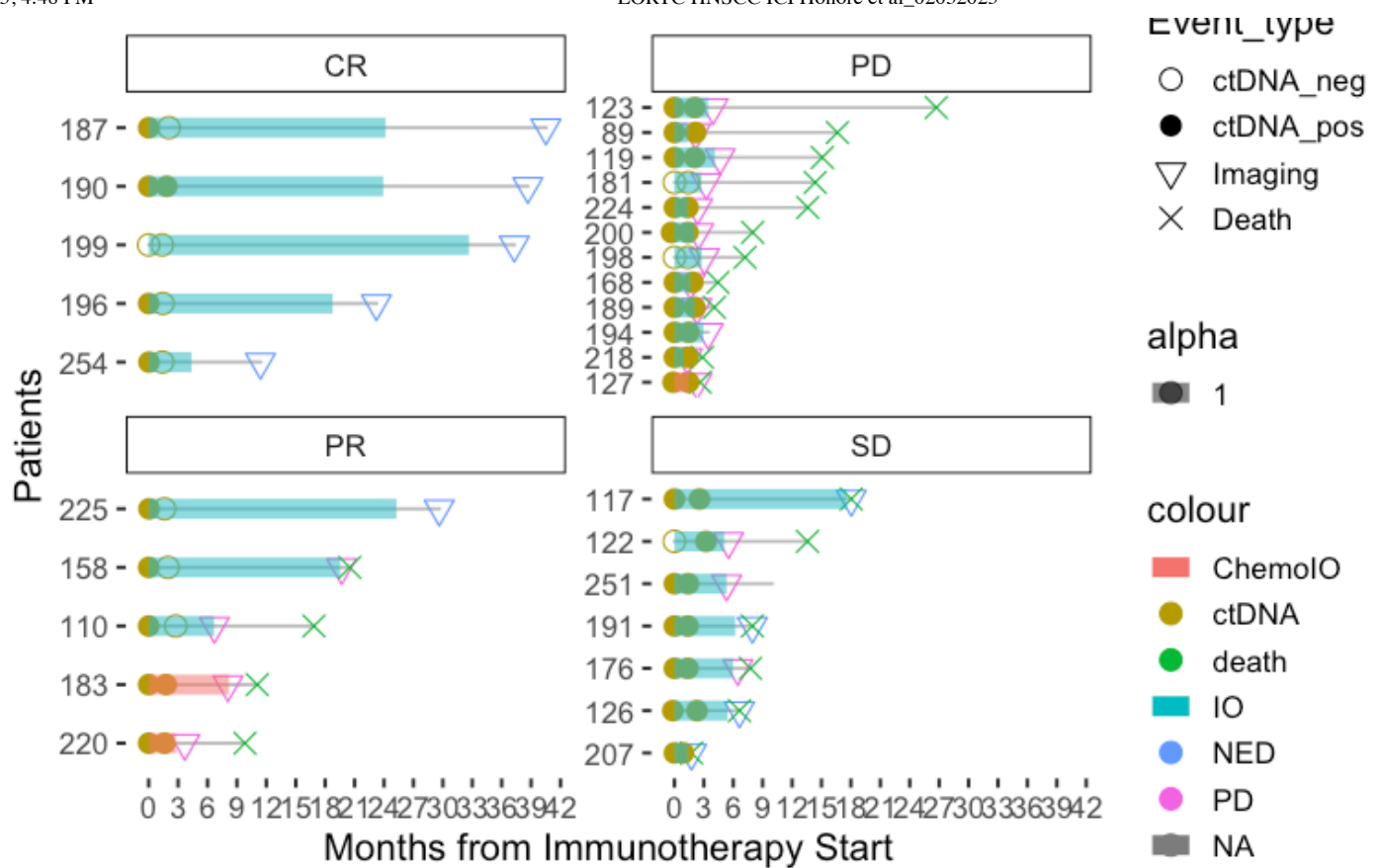


Hide

```

instage_df,
Name',
tart.months',
d.months',
x_type',
= 1.0)
oplot_ev2 <- oplot_ev1.1 + swimmer_lines(df_lines=cl
id='Patient
start='Tx_s
end='Tx_en
name_col='T
size=3.5,
name_alpha
oplot_ev2 <- oplot_ev2 + guides(linetype = guide_leg
end(override.aes = list(size = 5, color = "black"))
oplot_ev2

```

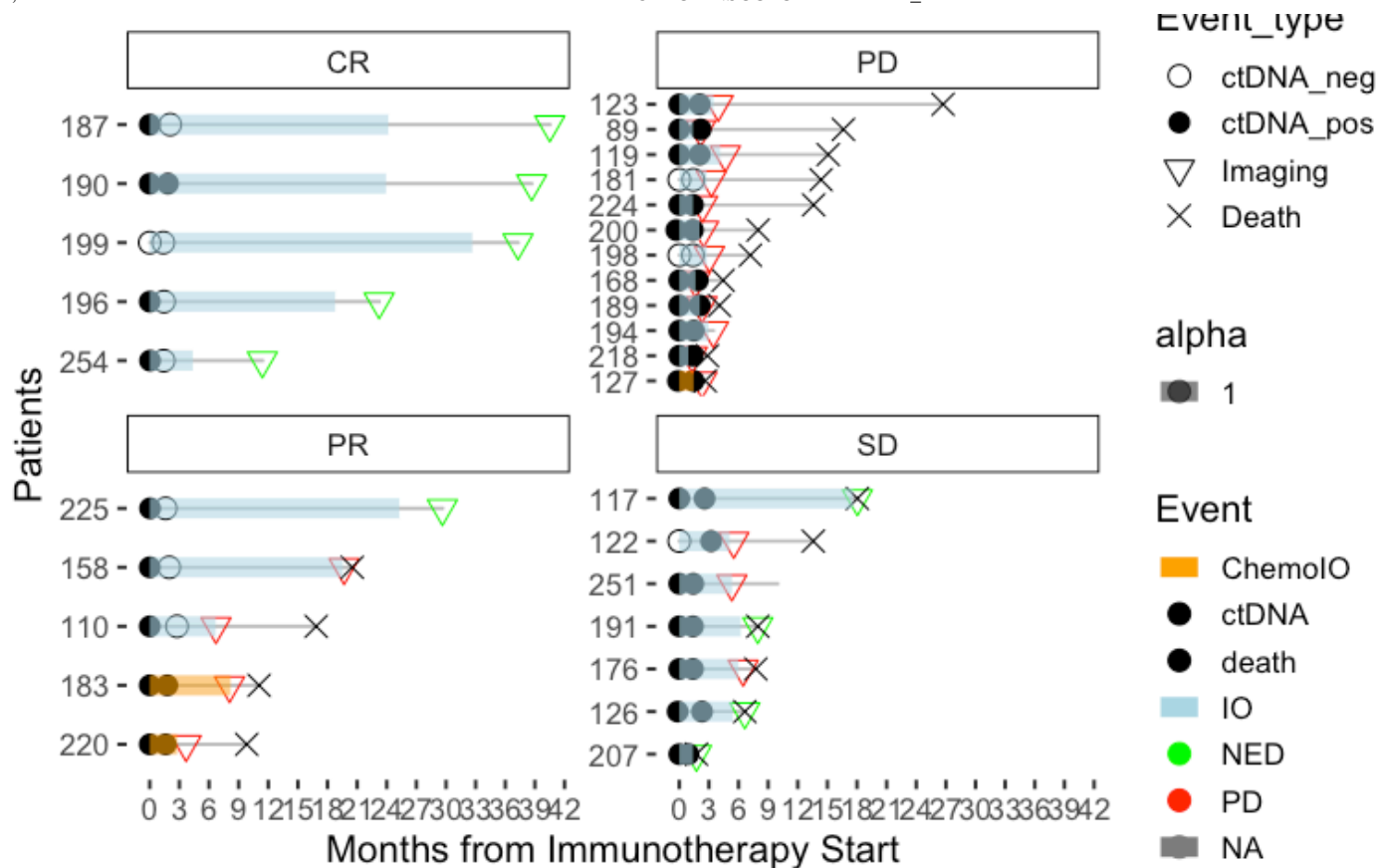



Hide

```

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)

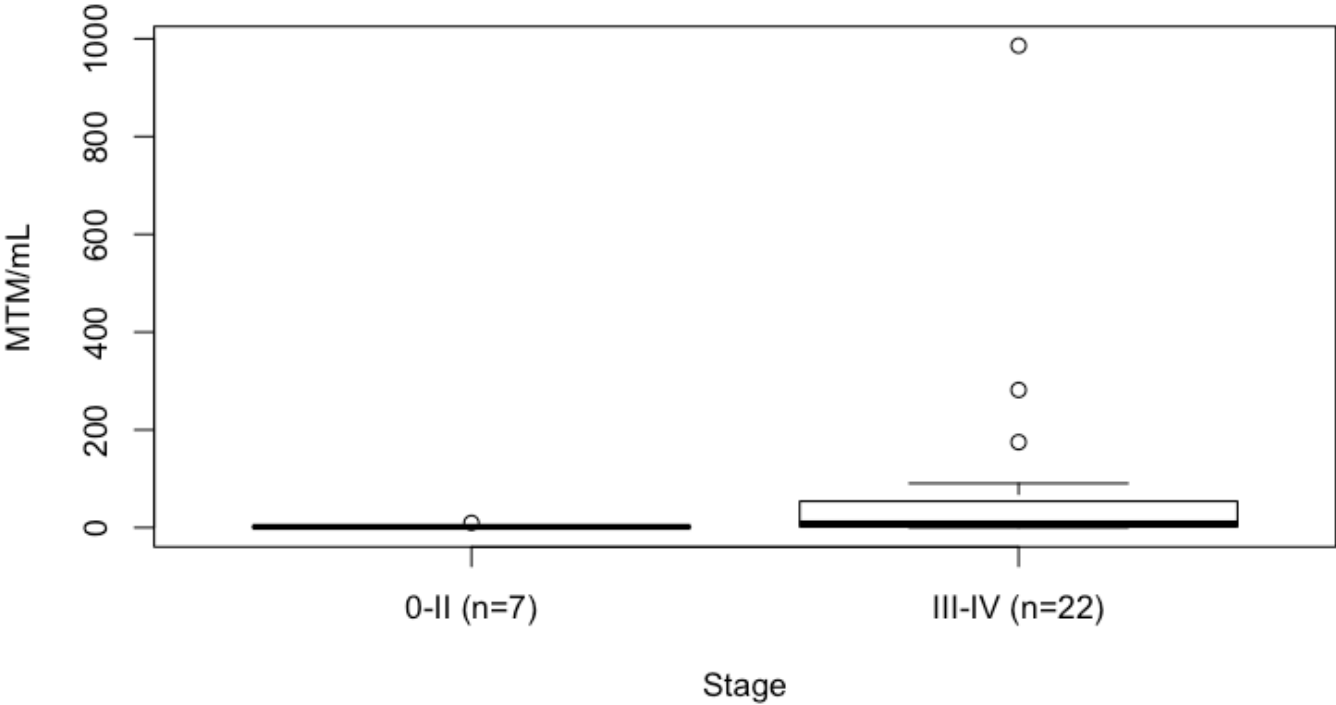
tally(~cStage, data=circ_data, margins = TRUE)
```

```
cStage
0-II III-IV Total
7 22 29
```

Hide

```
circ_data$cStage <- factor(circ_data$cStage, levels = c("0-II", "III-IV"), labels = c("0-II (n=7)", "III-IV (n=22)"))
boxplot(ctDNA.Base.MTM~cStage, data=circ_data, main="ctDNA pre-treatment MTM - Stage", x
lab="Stage", ylab="MTM/mL", col="white", border="black")
```

ctDNA pre-treatment MTM - Stage



Hide

```
m1<-wilcox.test(ctDNA.Base.MTM ~ cStage, data=circ_data, na.rm=TRUE, exact=FALSE, conf.i
nt=TRUE)
print(m1)
```

Wilcoxon rank sum test with continuity correction

```
data: ctDNA.Base.MTM by cStage
W = 39.5, p-value = 0.05896
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -5.19000e+01  4.27605e-05
sample estimates:
difference in location
 -6.799975
```

Hide

```
tally(~Inclusion.status, data=circ_data, margins = TRUE)
```

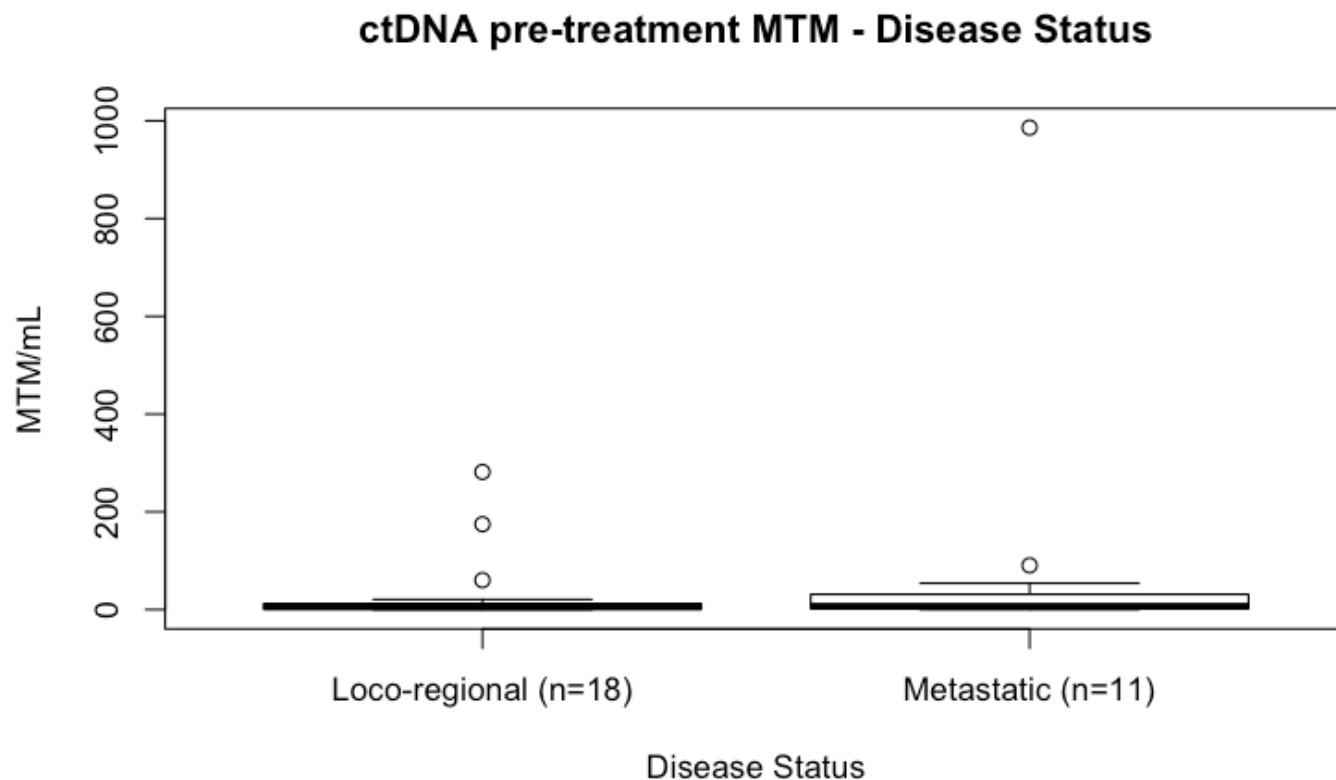
Inclusion.status		
Loco-regional	Metastatic	Total
18	11	29

Hide

```

circ_data$Inclusion.status <- factor(circ_data$Inclusion.status, levels = c("Loco-regional", "Metastatic"), labels = c("Loco-regional (n=18)", "Metastatic (n=11)"))
boxplot(ctDNA.Base.MTM~Inclusion.status, data=circ_data, main="ctDNA pre-treatment MTM - Disease Status", xlab="Disease Status", ylab="MTM/mL", col="white",border="black")

```



Hide

```

m2<-wilcox.test(ctDNA.Base.MTM ~ Inclusion.status, data=circ_data, na.rm=TRUE, exact=FAL
SE, conf.int=TRUE)
print(m2)

```

Wilcoxon rank sum test with continuity correction

data: ctDNA.Base.MTM by Inclusion.status

W = 93.5, p-value = 0.8219

alternative hypothesis: true location shift is not equal to 0

95 percent confidence interval:

-8.399949 6.899953

sample estimates:

difference in location

-0.3727674

Hide

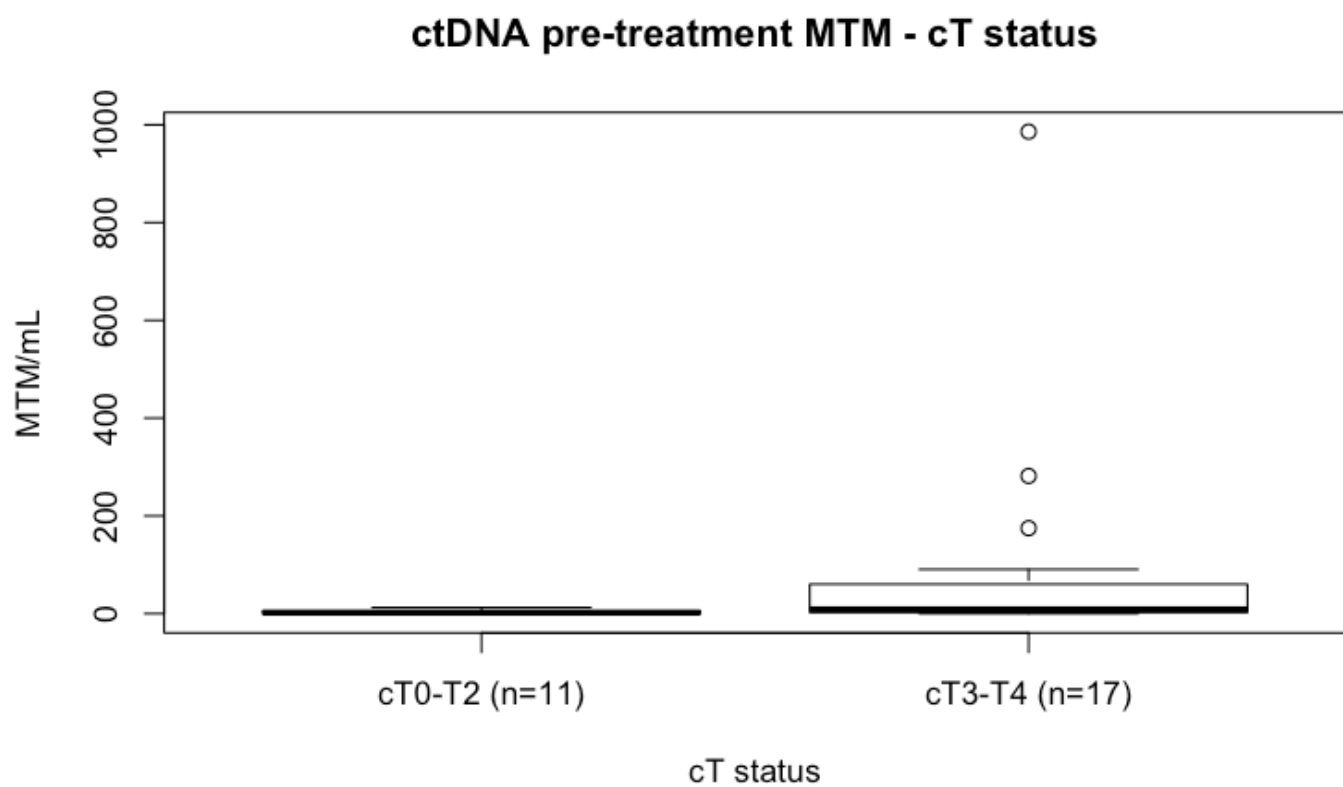
```
tally(~cT.Status, data=circ_data, margins = TRUE)
```

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 status", xlab="cT status", ylab="MTM/mL", col="white",border="black")
```


[Hide](#)

```
m3<-wilcox.test(ctDNA.Base.MTM ~ cT.Status, data=circ_data, na.rm=TRUE, exact=FALSE, conf.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

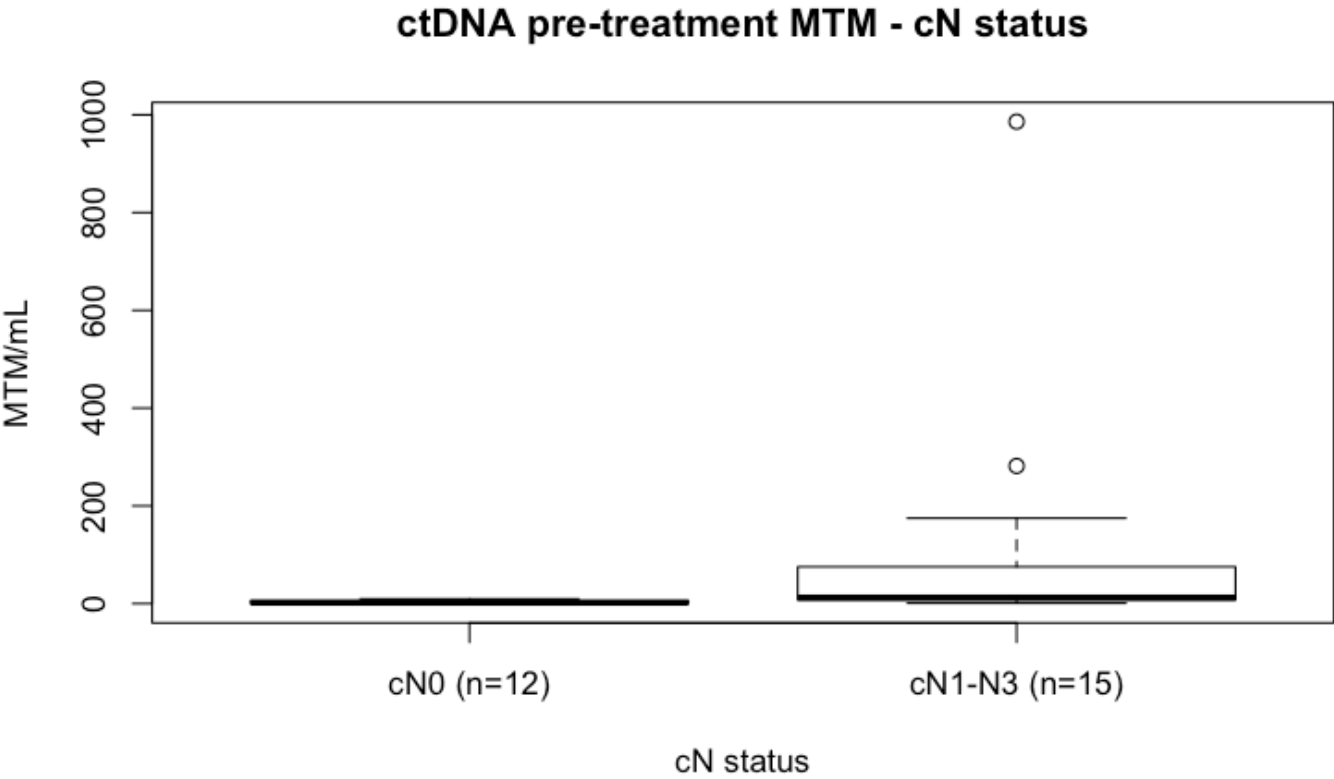
Hide

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 status", xlab="cN status", ylab="MTM/mL", col="white",border="black")



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
```

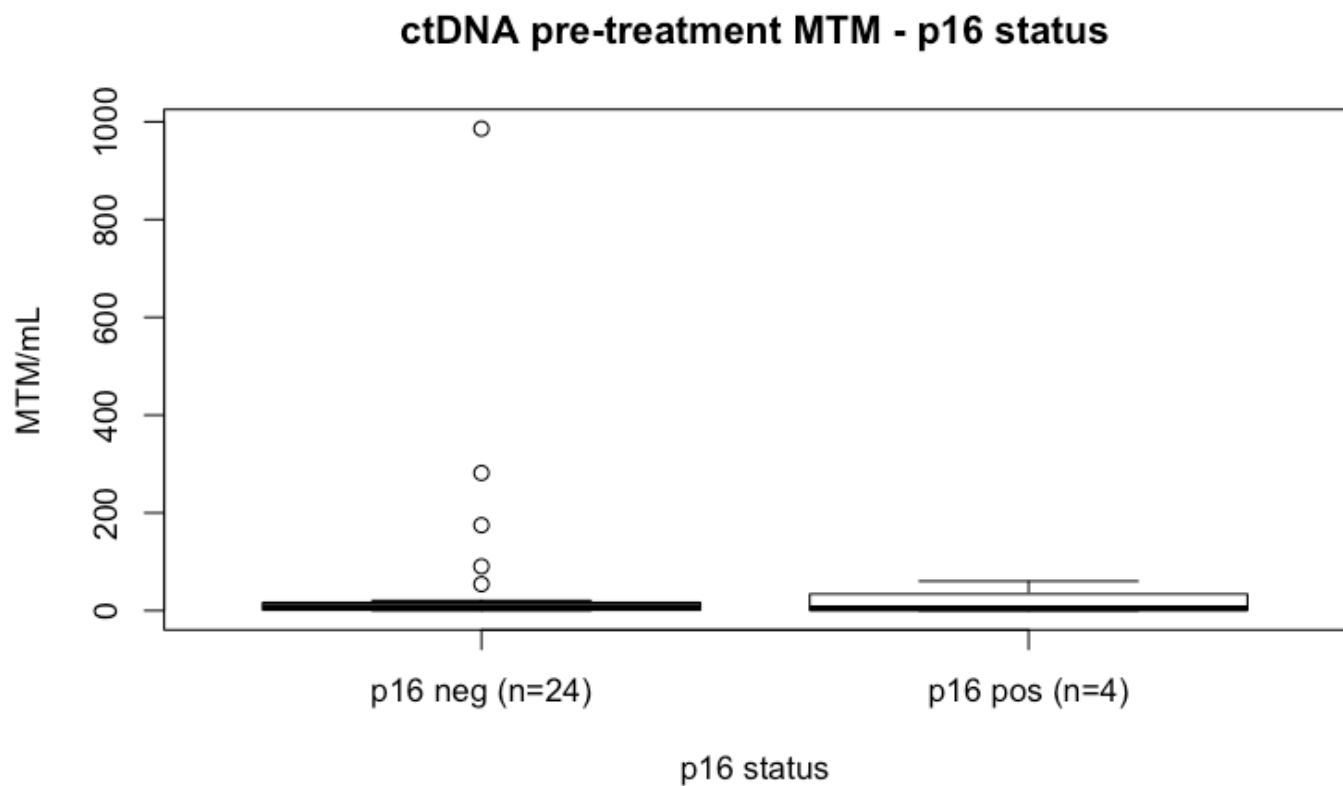
Hide

```
tally(~p16.status, data=circ_data, margins = TRUE)
```

```
p16.status
Negative Positive Unknown Total
      24       4       1     29
```

Hide

```
circ_data$p16.status <- factor(circ_data$p16.status, levels = c("Negative","Positive"),
labels = c("p16 neg (n=24)","p16 pos (n=4)"))
boxplot(ctDNA.Base.MTM~p16.status, data=circ_data, main="ctDNA pre-treatment MTM - p16 s
tatus", xlab="p16 status", ylab="MTM/mL", col="white",border="black")
```



Hide

```
m5<-wilcox.test(ctDNA.Base.MTM ~ p16.status, data=circ_data, na.rm=TRUE, exact=FALSE, co
nf.int=TRUE)
print(m5)
```

Wilcoxon rank sum test with continuity correction

```
data: ctDNA.Base.MTM by p16.status
W = 55.5, p-value = 0.6453
alternative hypothesis: true location shift is not equal to 0
95 percent confidence interval:
 -47.89994  46.29998
sample estimates:
difference in location
      0.8901786
```

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

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",]

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

Hide

```
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

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_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

Hide

```
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

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_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, data = 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)
```

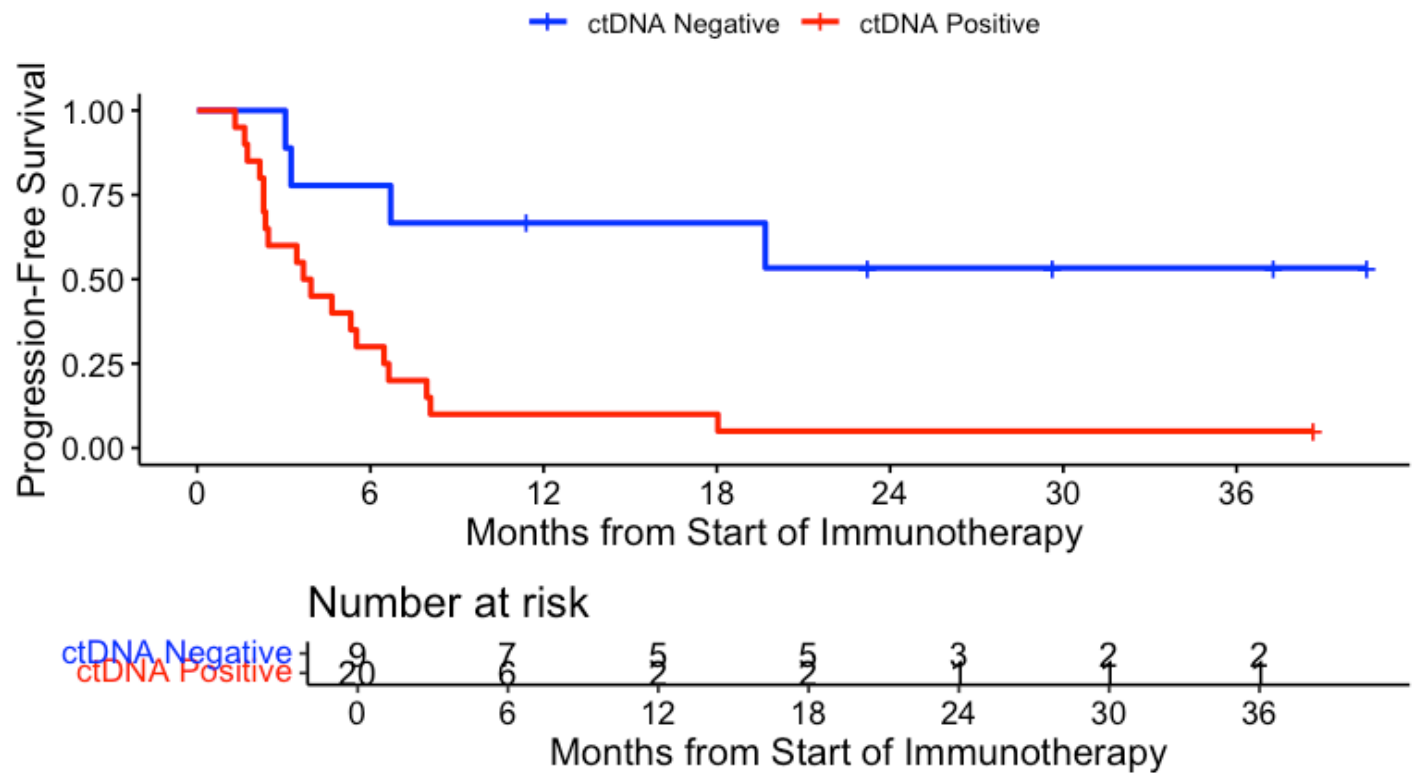
ctDNA.postTx <chr>	Total <int>	Events <int>	Fraction <dbl>	Percentage <dbl>
NEGATIVE	9	4	0.4444444	44.44444
POSITIVE	20	19	0.9500000	95.00000

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 = TRUE,
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



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```
summary(KM_curve, times= c(0, 12, 24, 36))
```

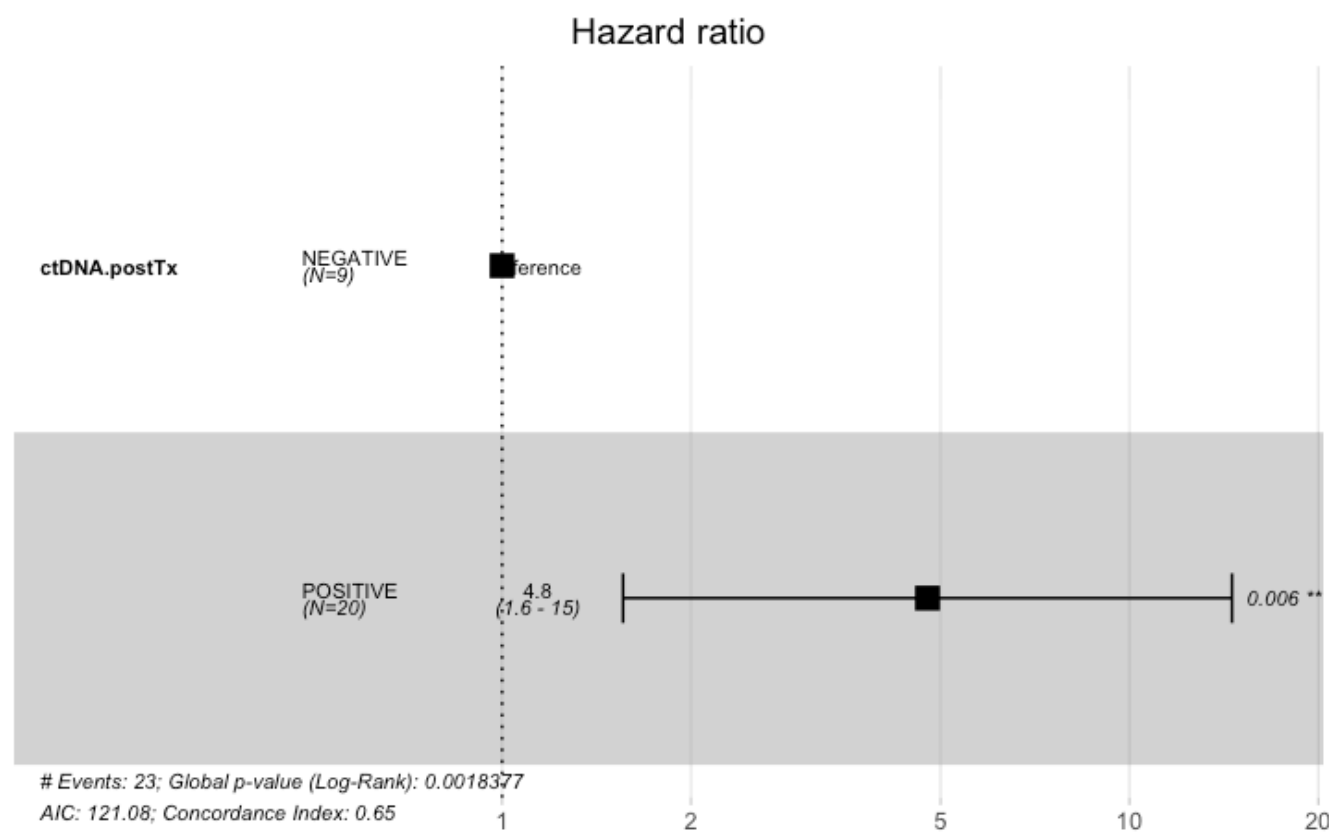
```
Call: survfit(formula = surv_object ~ ctDNA.postTx, data = circ_data,
  conf.int = 0.95, conf.type = "log-log")
```

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	

Hide

```
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)
```



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

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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 = 4.77 (1.56-14.58); p = 0.006"
```

Hide

```
circ_data$ctDNA.postTx <- factor(circ_data$ctDNA.postTx, levels = c("NEGATIVE", "POSITIVE"), 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)
```

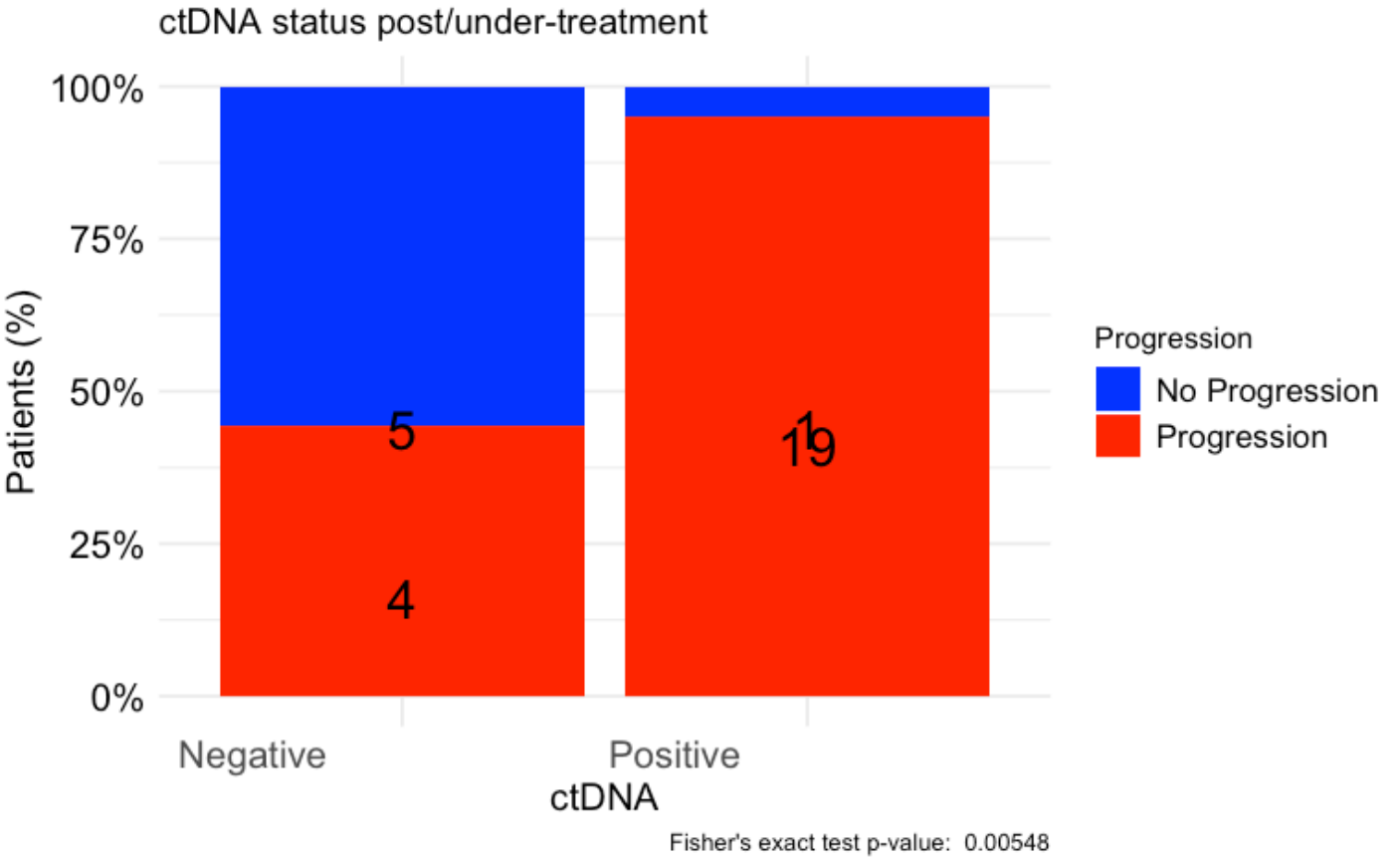
	No Progression	Progression
Negative	5	4
Positive	1	19

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 = "black", 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")) + # 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 size
        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 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)
```

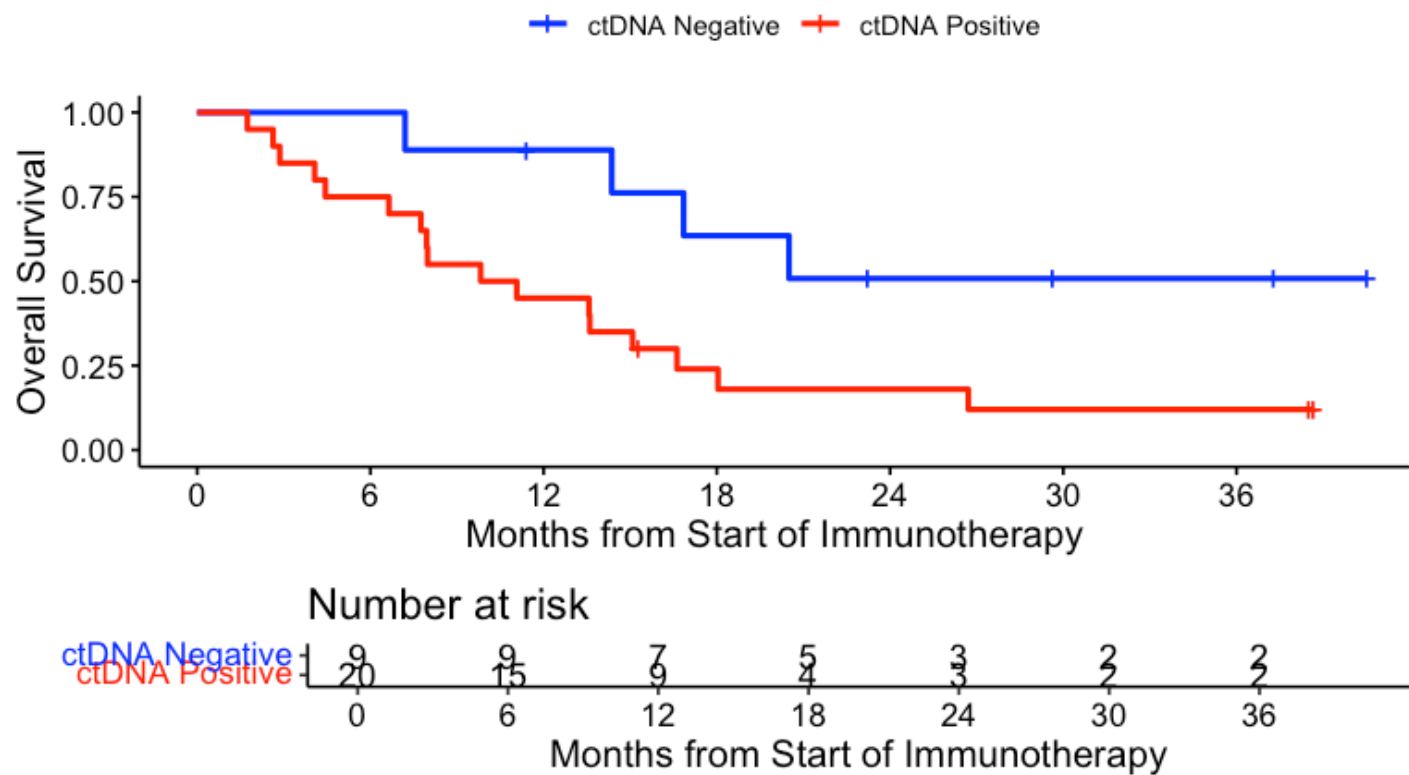
ctDNA.postTx	Total	Events	Fraction	Percentage
<chr>	<int>	<int>	<dbl>	<dbl>
NEGATIVE	9	4	0.4444444	44.44444
POSITIVE	20	17	0.8500000	85.00000

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



Hide

```
summary(KM_curve, times= c(12, 24, 36))
```

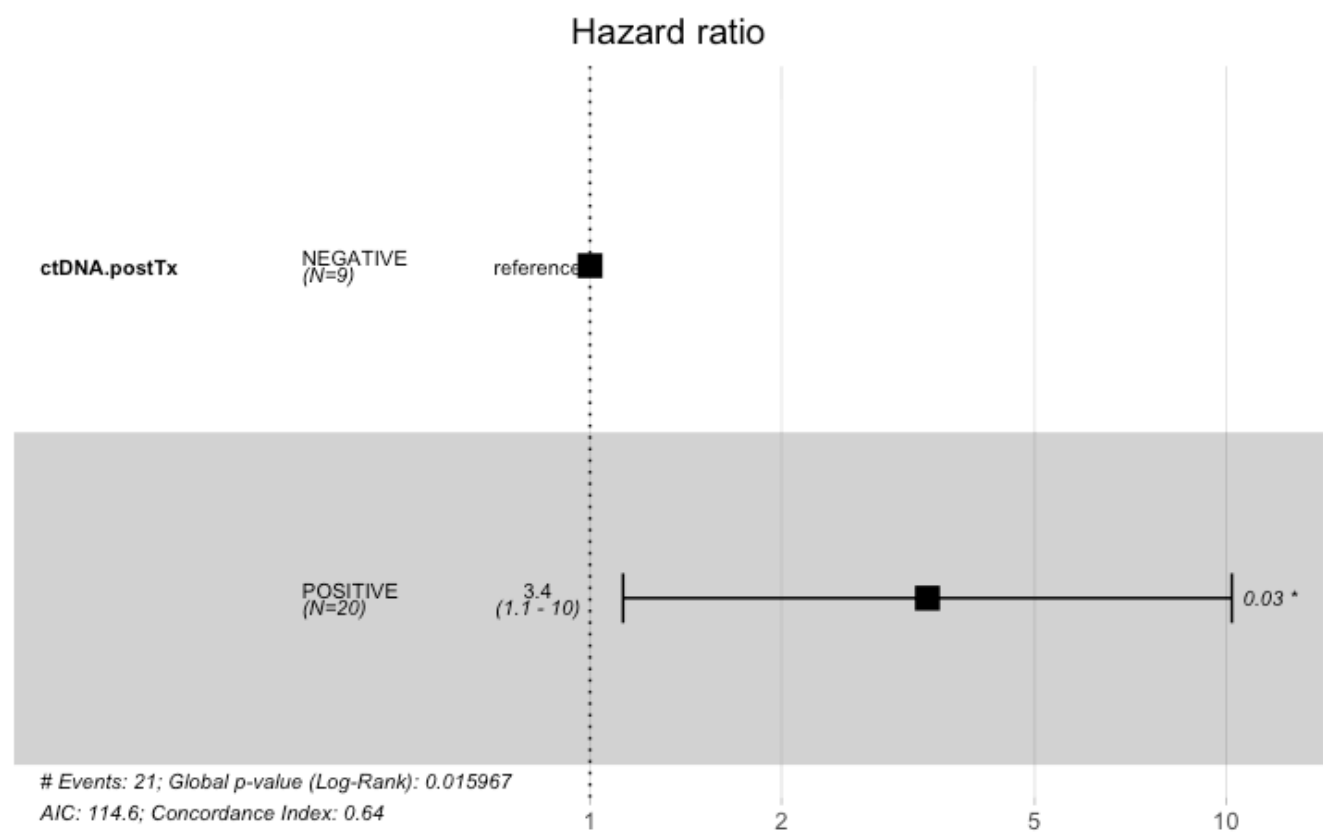
```
Call: survfit(formula = surv_object ~ ctDNA.postTx, data = circ_data,
  conf.int = 0.95, conf.type = "log-log")
```

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

Hide

```
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)
```



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)
```

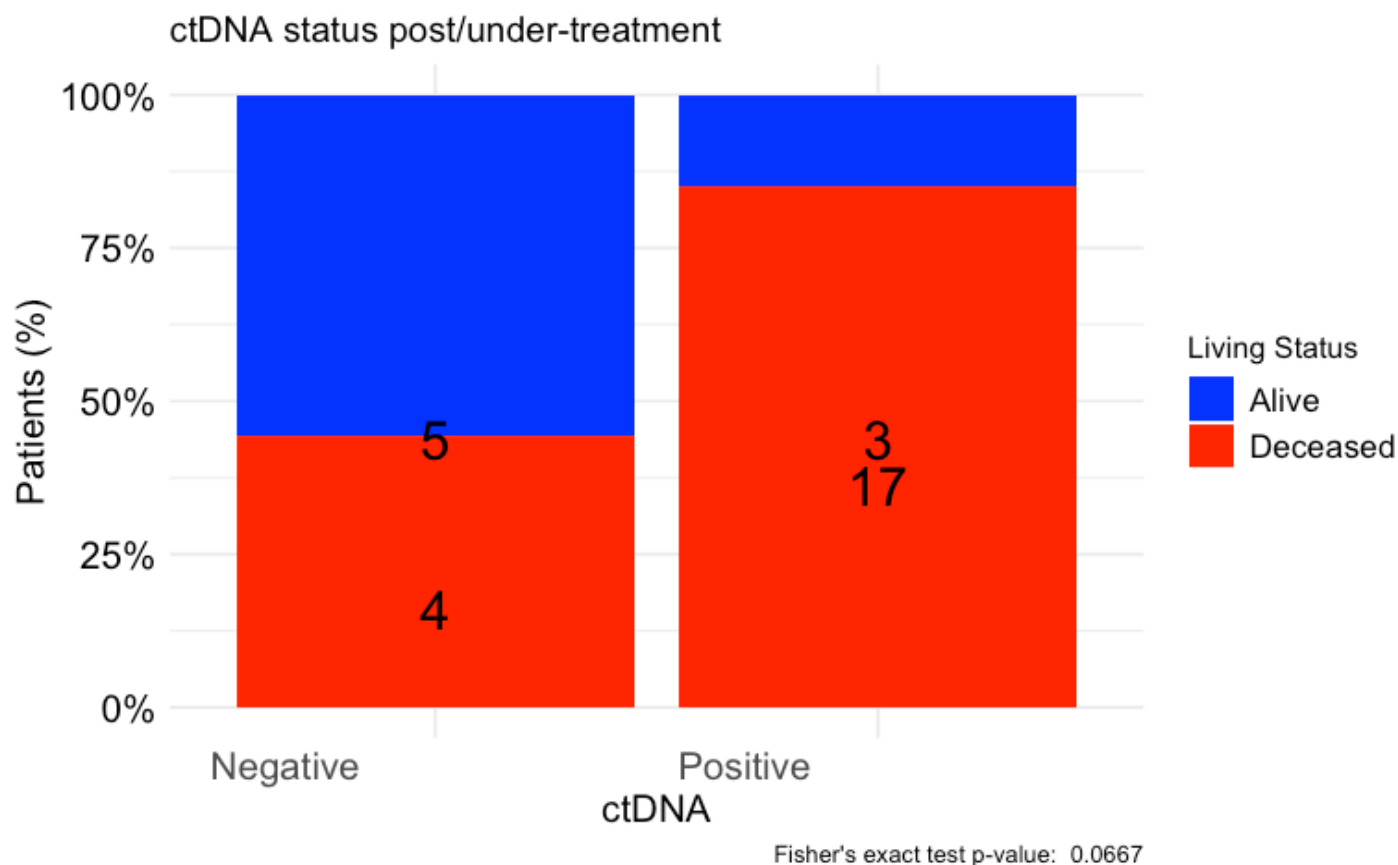
	Alive	Deceased
Negative	5	4
Positive	3	17

[Hide](#)

```

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 = "black", 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 size
        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

```



#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", "POSITIVE"), 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)
```

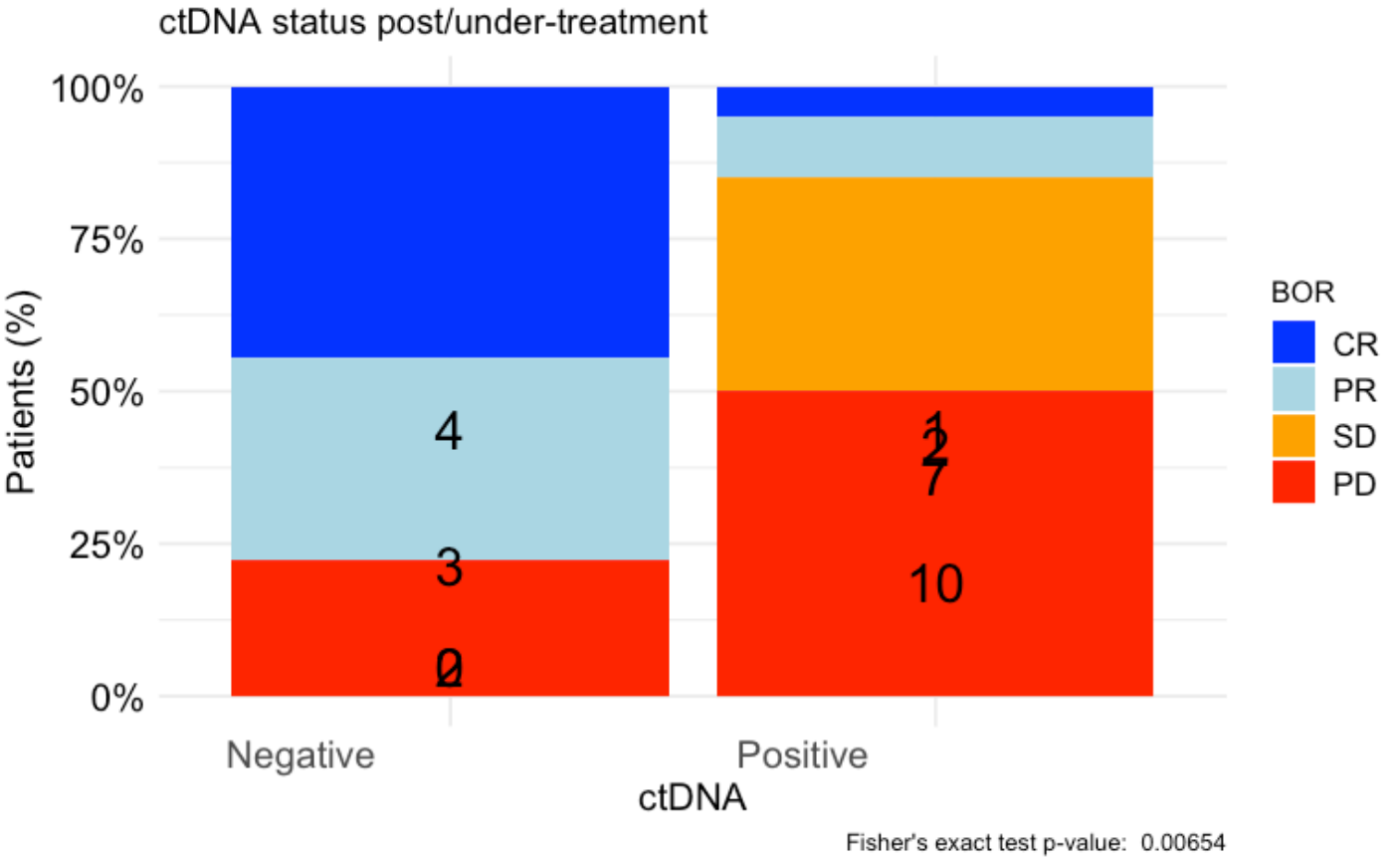
	CR	PR	SD	PD
Negative	4	3	0	2
Positive	1	2	7	10

[Hide](#)

```

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 = "black", 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 size
        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

```



#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 = circ_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

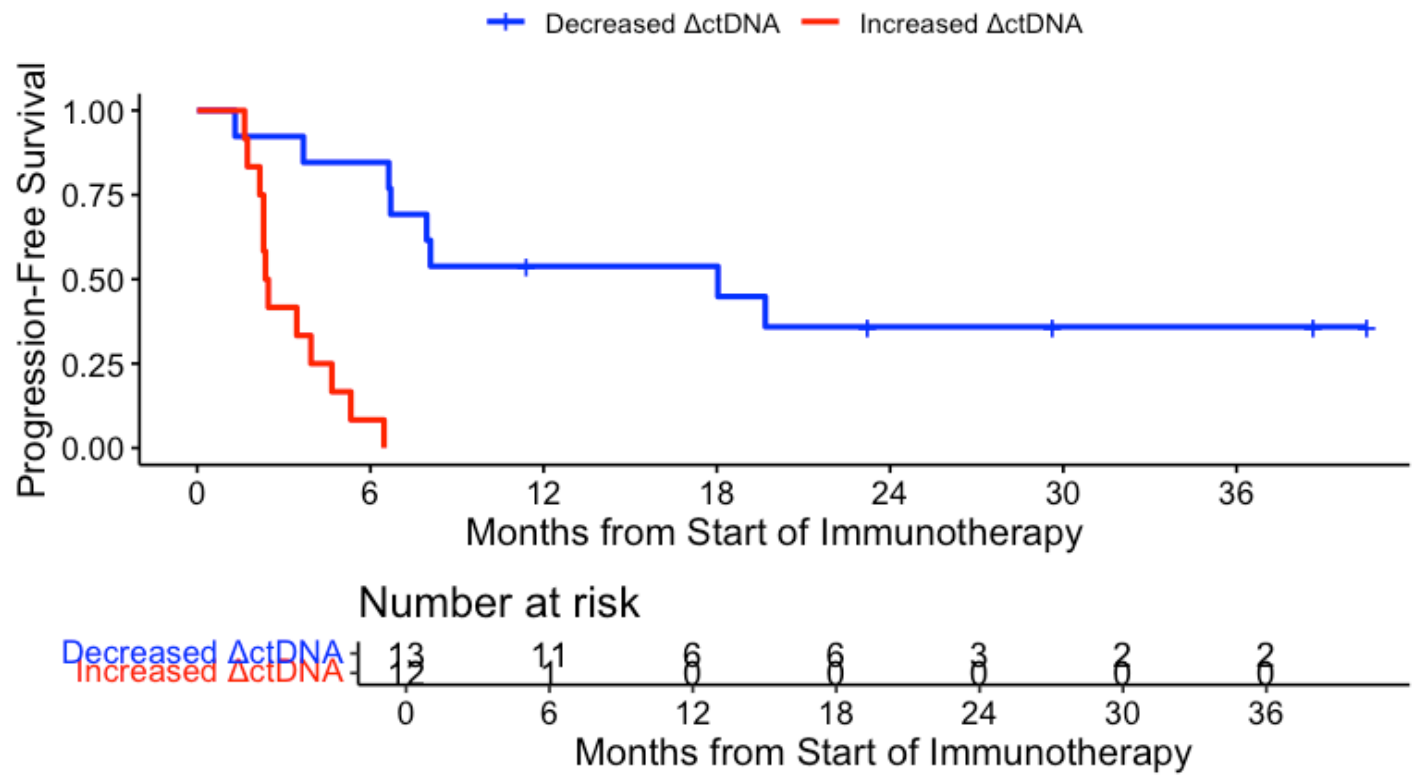
```
event_summary <- circ_data %>%
  group_by( $\Delta$ ctDNA) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

Δ ctDNA	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 ~  $\Delta$ ctDNA, 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, palette=c("blue","red"), title="PFS - ctDNA Kinetics Post/Under treatment", ylab= "Progression-Free Survival", xlab="Months from Start of Immunotherapy", legend.labs=c("Decreased  $\Delta$ ctDNA", "Increased  $\Delta$ ctDNA"), legend.title="")
```

PFS - ctDNA Kinetics Post/Under treatment



Hide

```
summary(KM_curve, times= c(0, 12, 24, 36))
```

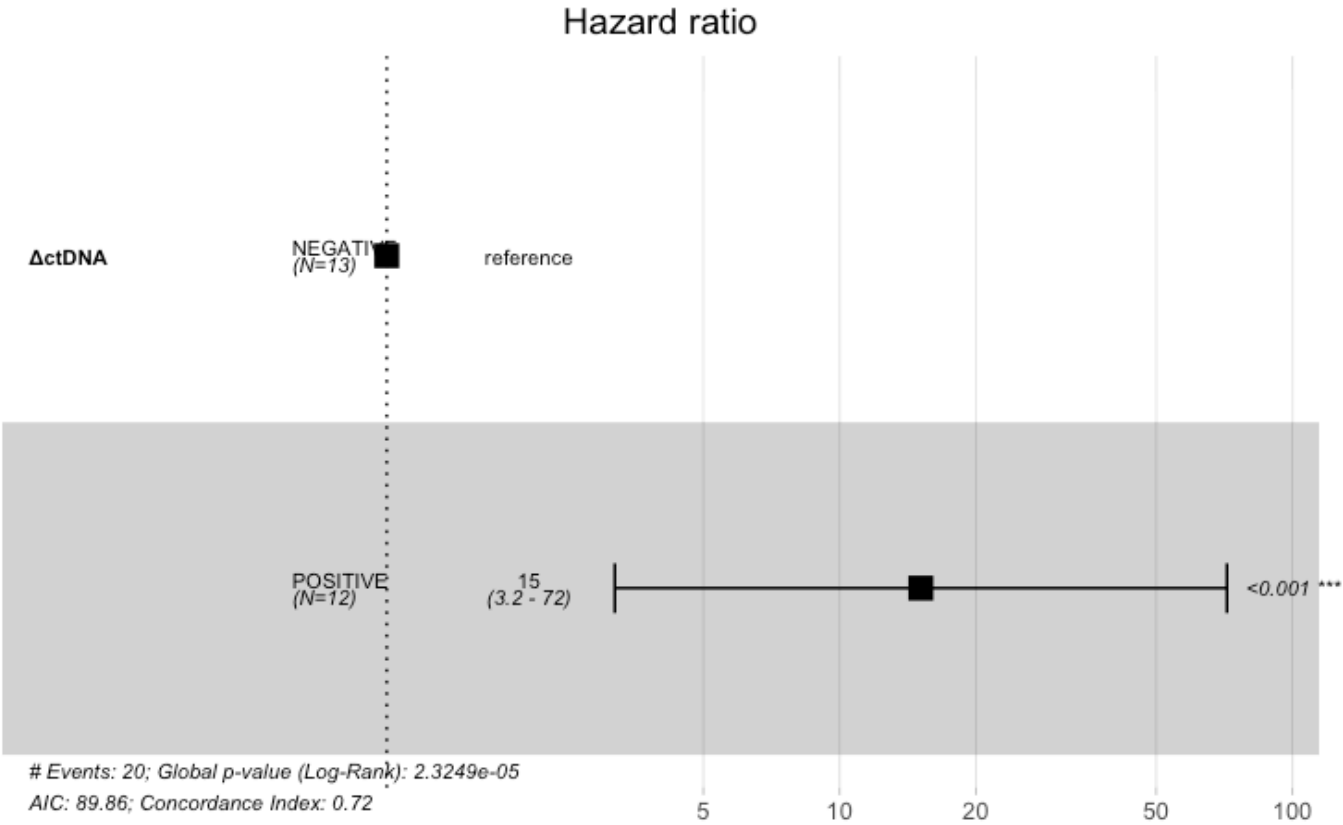
Call: `survfit(formula = surv_object ~ Δ ctDNA, data = circ_data, conf.int = 0.95, conf.type = "log-log")`

Δ ctDNA=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	

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

Hide

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



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_CI, 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)
```

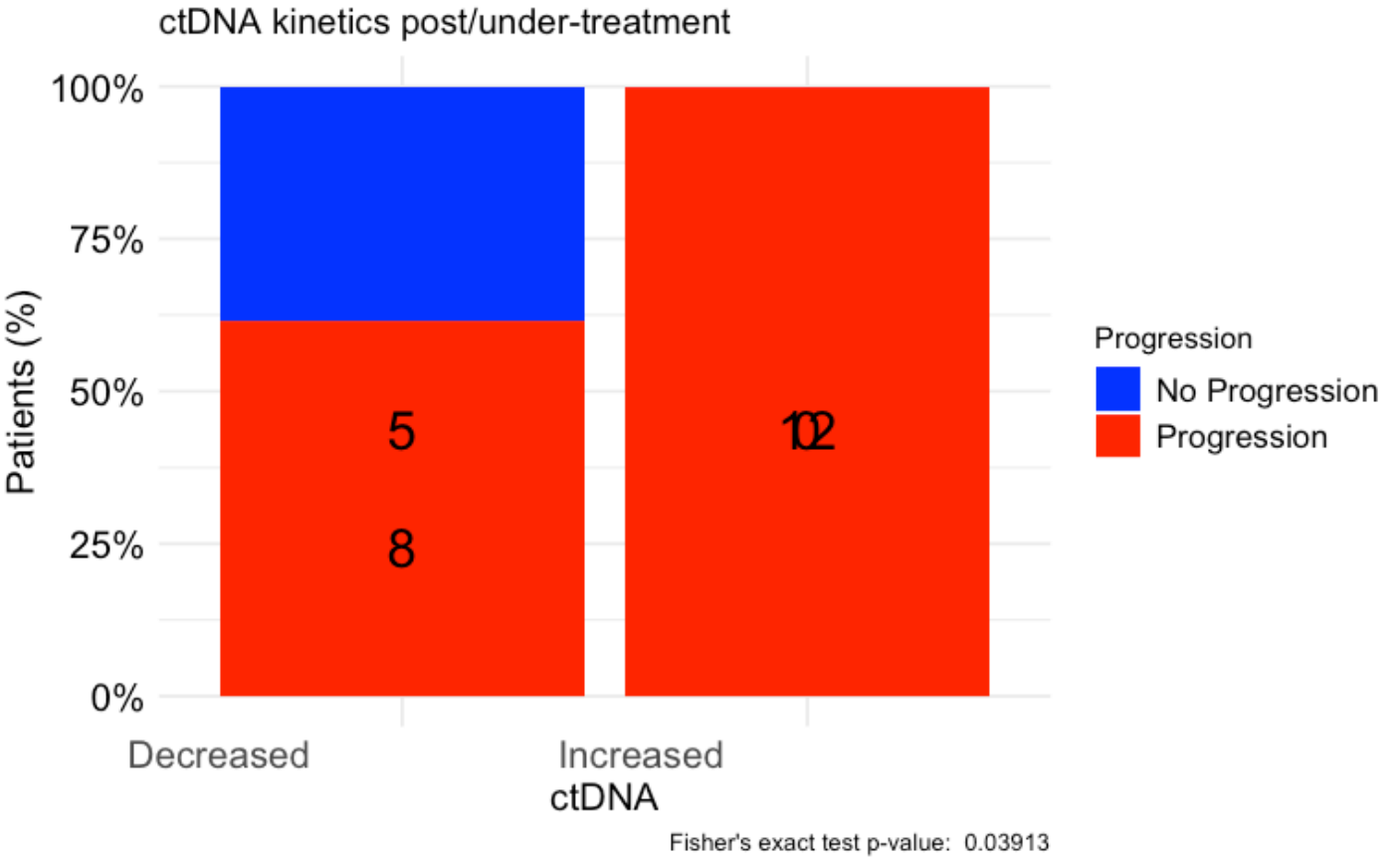
	No Progression	Progression
Decreased	5	8
Increased	0	12

[Hide](#)


```

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 = "black",
    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

```



#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

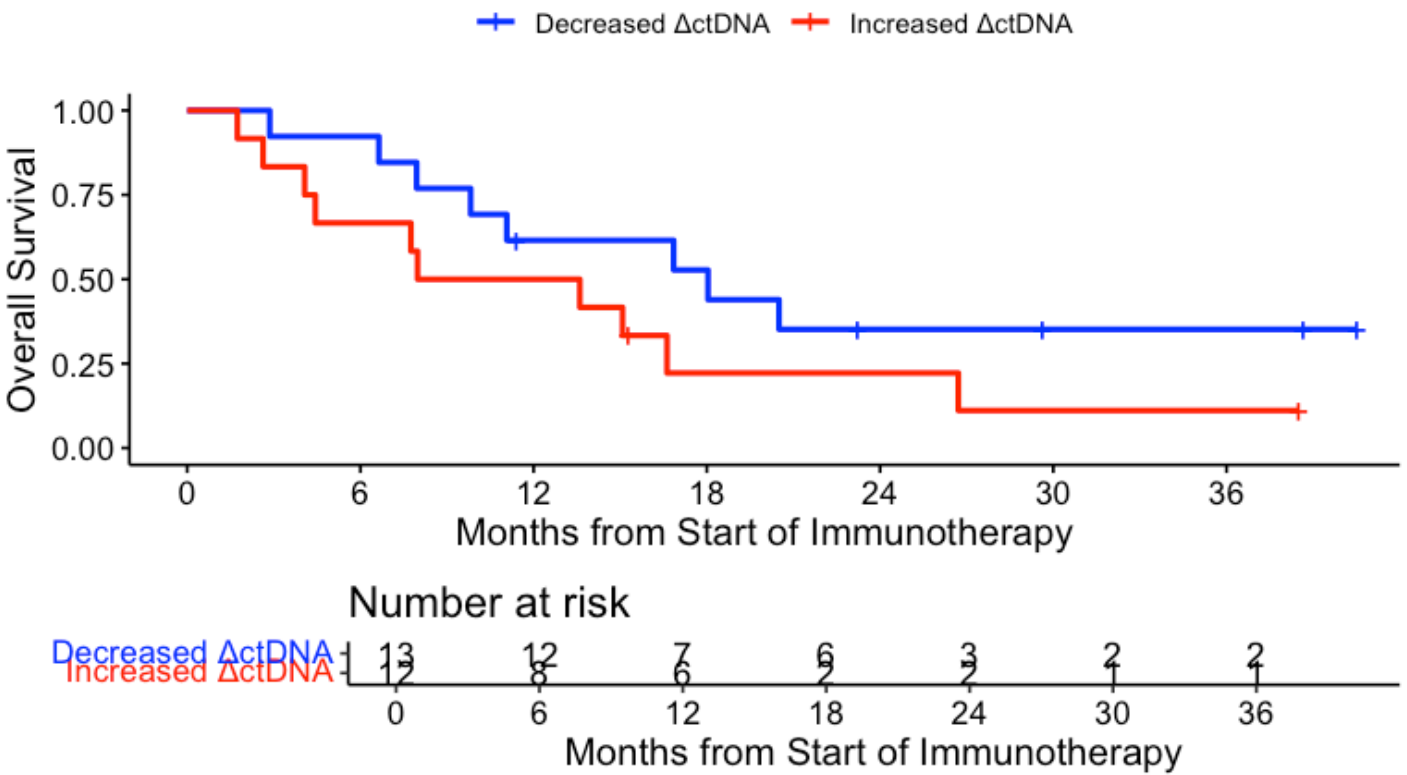
```
event_summary <- circ_data %>%
  group_by(ΔctDNA) %>%
  summarise(
    Total = n(),
    Events = sum(OS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

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 = TRUE, break.time.by=6, palette=c("blue","red"), title="OS - ctDNA Kinetics Post/Under treatment", ylab= "Overall Survival", xlab="Months from Start of Immunotherapy", legend.labs=c("Decreased ΔctDNA", "Increased ΔctDNA"), legend.title="")
```

OS - ctDNA Kinetics Post/Under treatment



Hide

```
summary(KM_curve, times= c(0, 12, 24, 36))
```

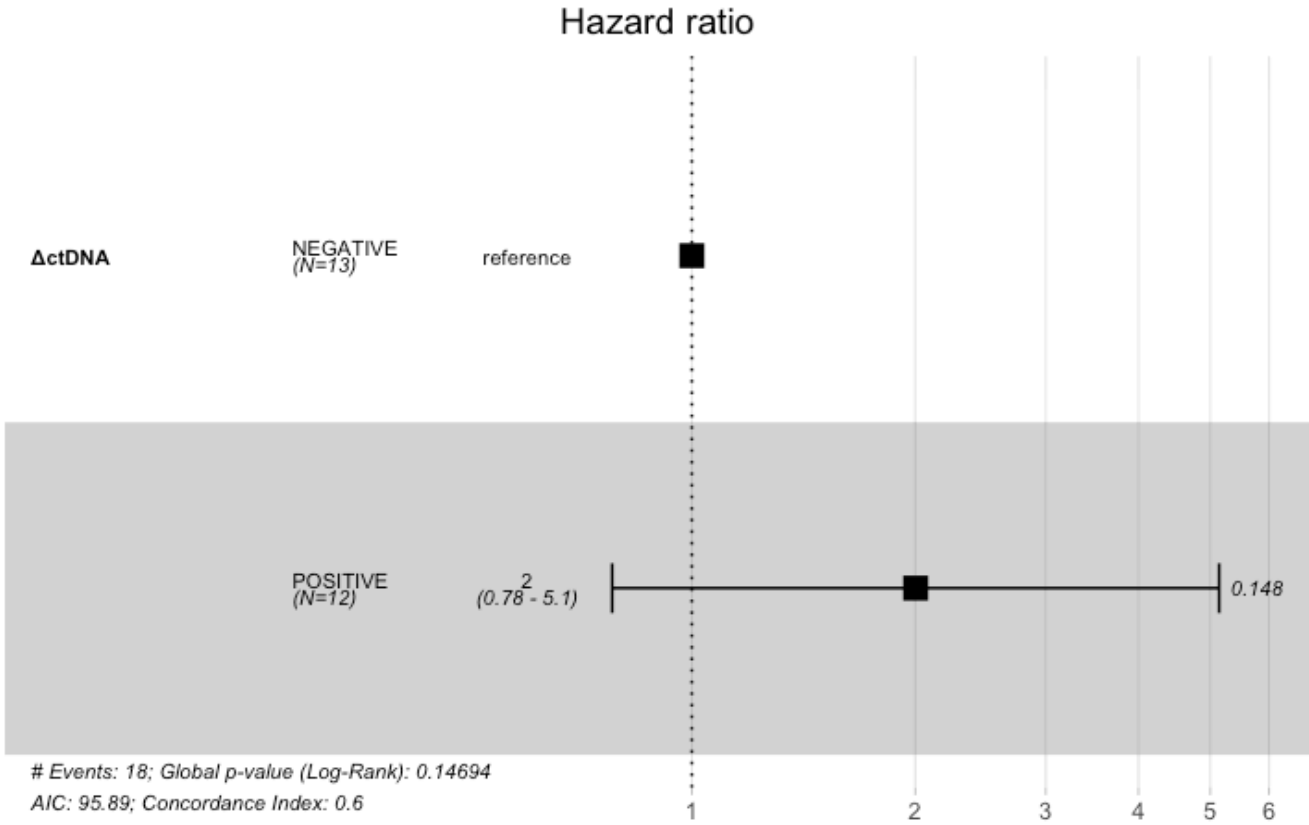
Call: `survfit(formula = surv_object ~ Δ ctDNA, data = circ_data, conf.int = 0.95, conf.type = "log-log")`

Δ ctDNA=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	

Δ ctDNA=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	

Hide

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



Hide

```
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

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_CI, 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)
```

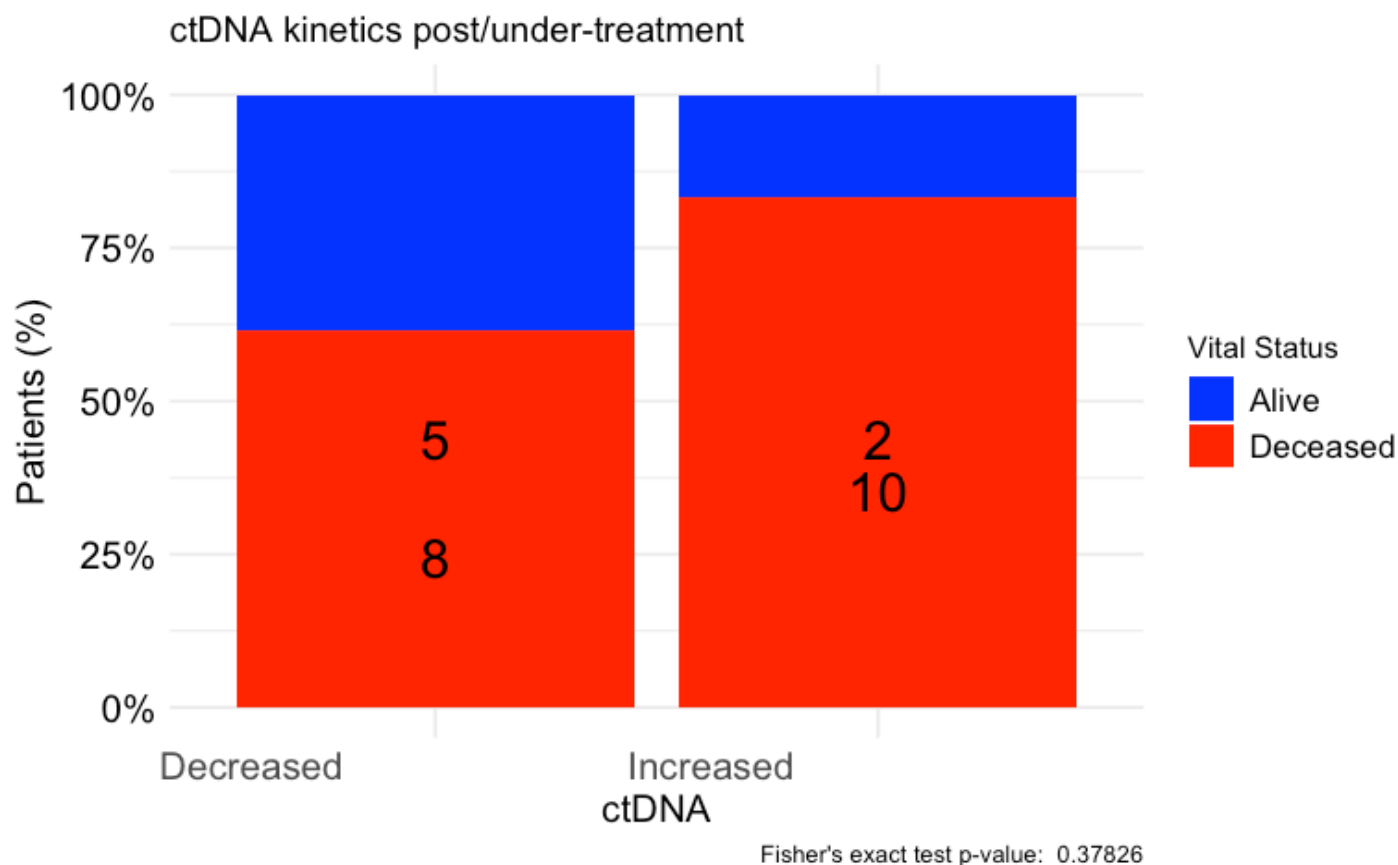
	Alive	Deceased
Decreased	5	8
Increased	2	10

[Hide](#)

```

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 = "black", 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 size
        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

```

#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)
```

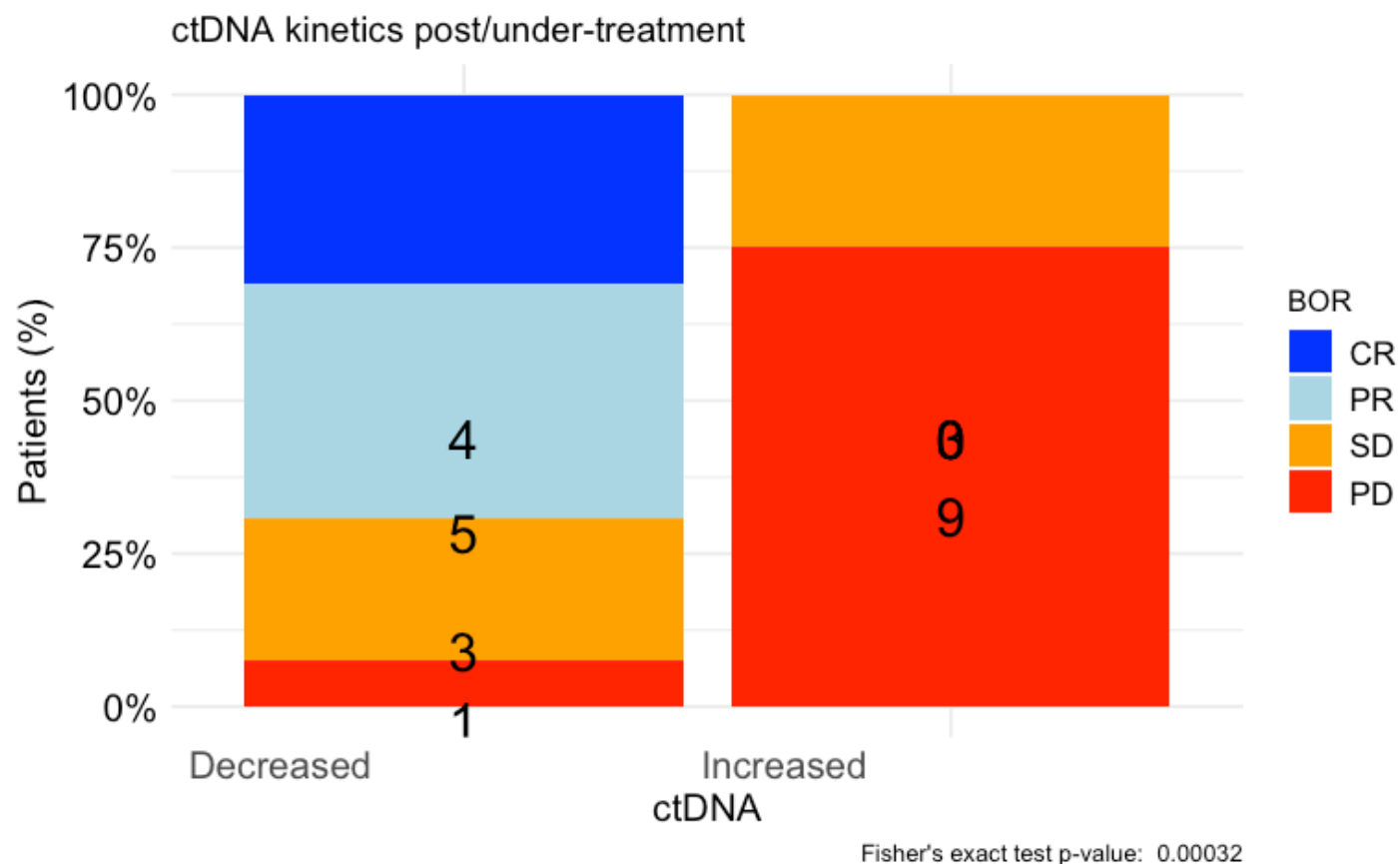
	CR	PR	SD	PD
Decreased	4	5	3	1
Increased	0	0	3	9

Hide

```

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 = "black",
    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 size
    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

```



#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 #first we create the variable for the ctDNA & NAC combina
tion, and we assign values
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)
```

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

	n	events	median	0.95LCL	0.95UCL
ctDNA.Dynamics=1	6	2	NA	19.68	NA
ctDNA.Dynamics=2	19	18	3.68	2.37	7.95

Hide

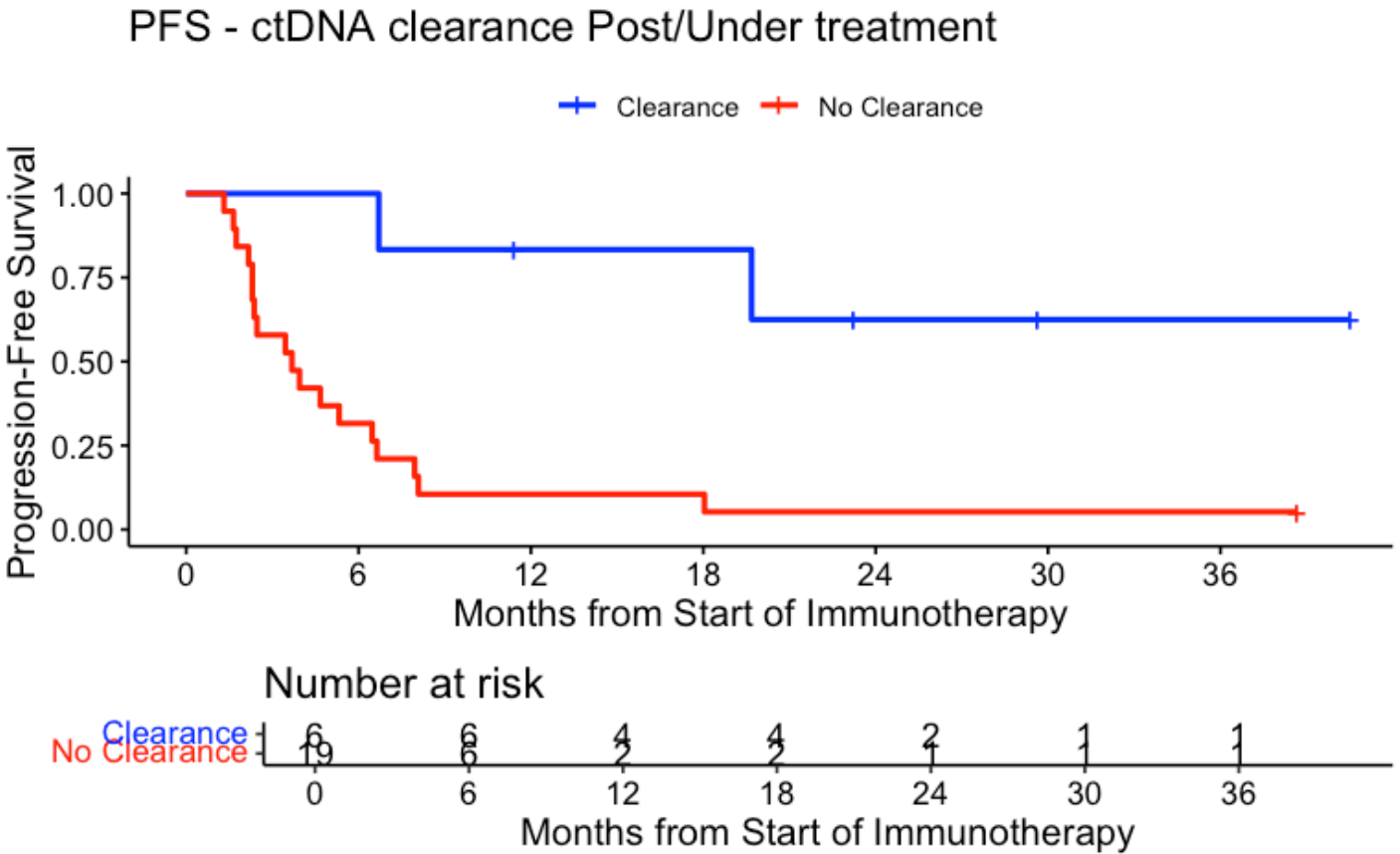
```
event_summary <- circ_data %>%
  group_by(ctDNA.Dynamics) %>%
  summarise(
    Total = n(),
    Events = sum(PFS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

ctDNA.Dynamics	Total	Events	Fraction	Percentage
<dbl>	<int>	<int>	<dbl>	<dbl>
1	6	2	0.3333333	33.33333
2	19	18	0.9473684	94.73684

2 rows

Hide

```
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.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=6, palette=c("blue","red"), title="PFS – ctDNA clearance Post/Under treatment", ylab= "Progression-Free Survival", xlab="Months from Start of Immunotherapy", legend.labs=c("Clearance", "No Clearance"), legend.title="")
```



Hide

```
summary(KM_curve, times= c(0, 12, 24, 36))
```

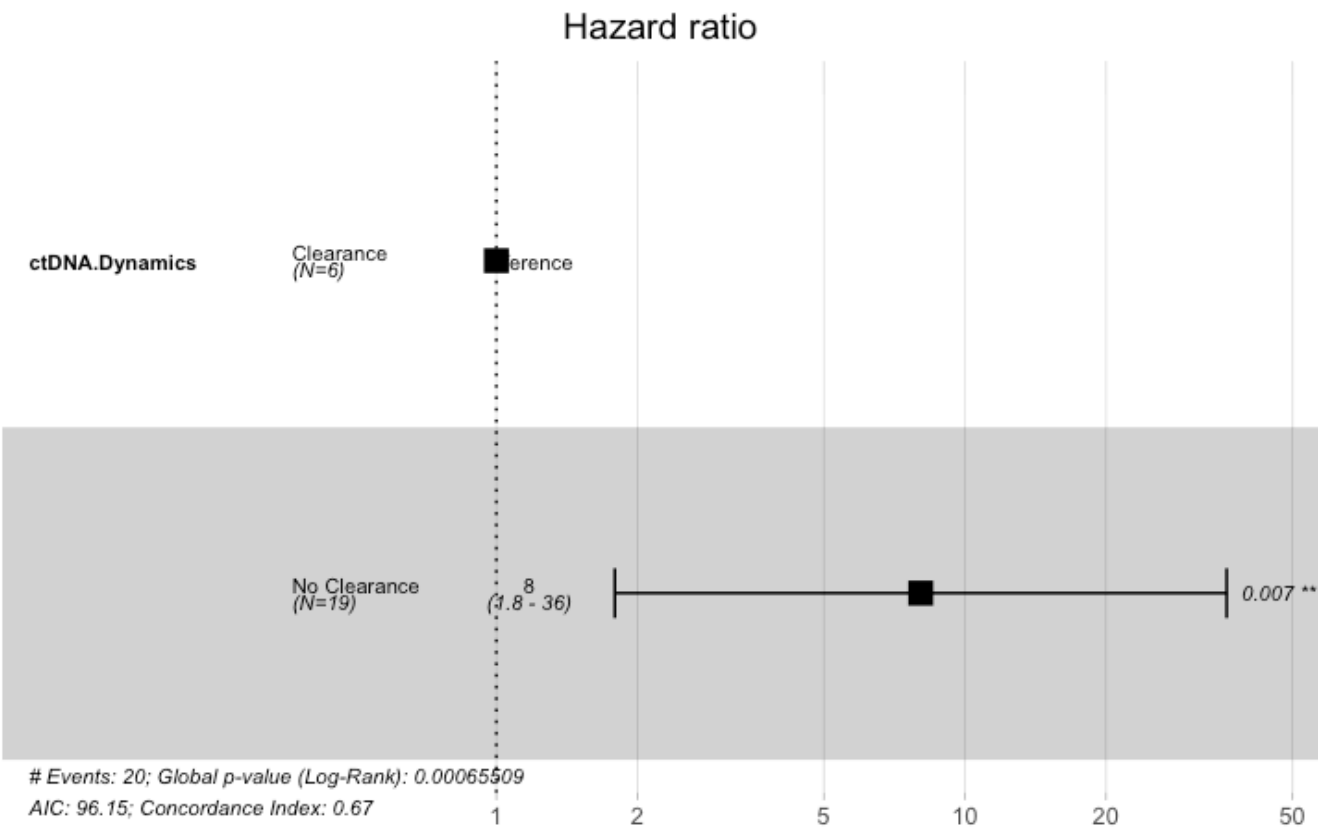
```
Call: survfit(formula = surv_object ~ ctDNA.Dynamics, data = circ_data,
  conf.int = 0.95, conf.type = "log-log")
```

ctDNA.Dynamics=1							
time	n.risk	n.event	survival	std.err	lower 95% CI	upper 95% CI	
0	6	0	1.000	0.000	1.000	1.000	
12	4	1	0.833	0.152	0.273	0.975	
24	2	1	0.625	0.213	0.142	0.893	
36	1	0	0.625	0.213	0.142	0.893	

ctDNA.Dynamics=2							
time	n.risk	n.event	survival	std.err	lower 95% CI	upper 95% CI	
0	19	0	1.0000	0.0000	1.00000	1.000	
12	2	17	0.1053	0.0704	0.01777	0.284	
24	1	1	0.0526	0.0512	0.00359	0.214	
36	1	0	0.0526	0.0512	0.00359	0.214	

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)
```



Hide

```
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_CI, 2), "); p = ", round(p_value, 3))
```

```
print(label_text)
```

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

[Hide](#)

```
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)
```

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 = 7.2505, df = 1, p-value = 0.007088
```

[Hide](#)

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

Fisher's Exact Test for Count Data

```
data: contingency_table
p-value = 0.005477
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 1.796359 1833.444857
sample estimates:
odds ratio
 27.59073
```

[Hide](#)

```
print(contingency_table)
```

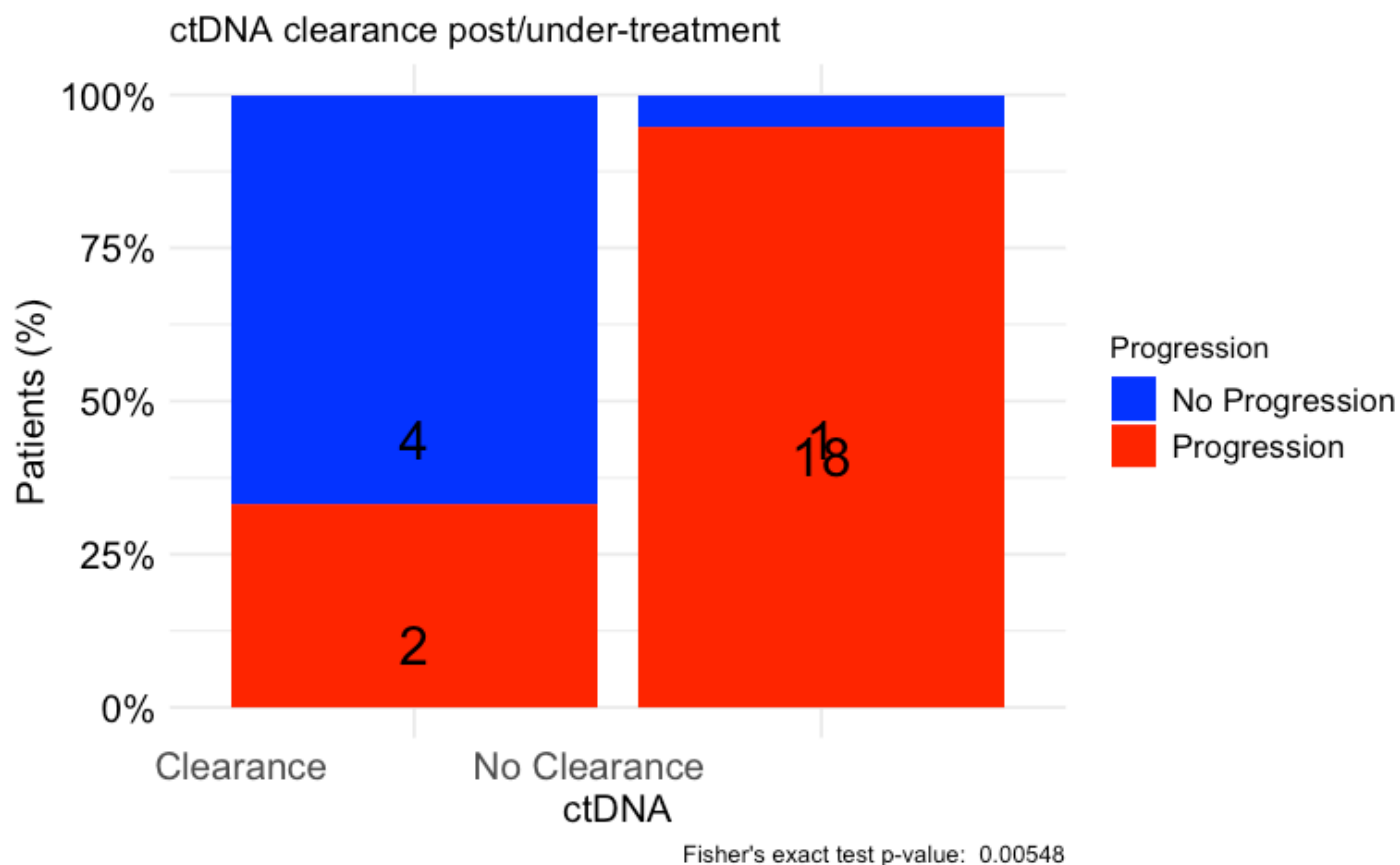
	No Progression	Progression
Clearance	4	2
No Clearance	1	18

[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 = "black", 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")) + # 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 size
        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

```
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 #first we create the variable for the ctDNA & NAC combina
tion, and we assign values
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

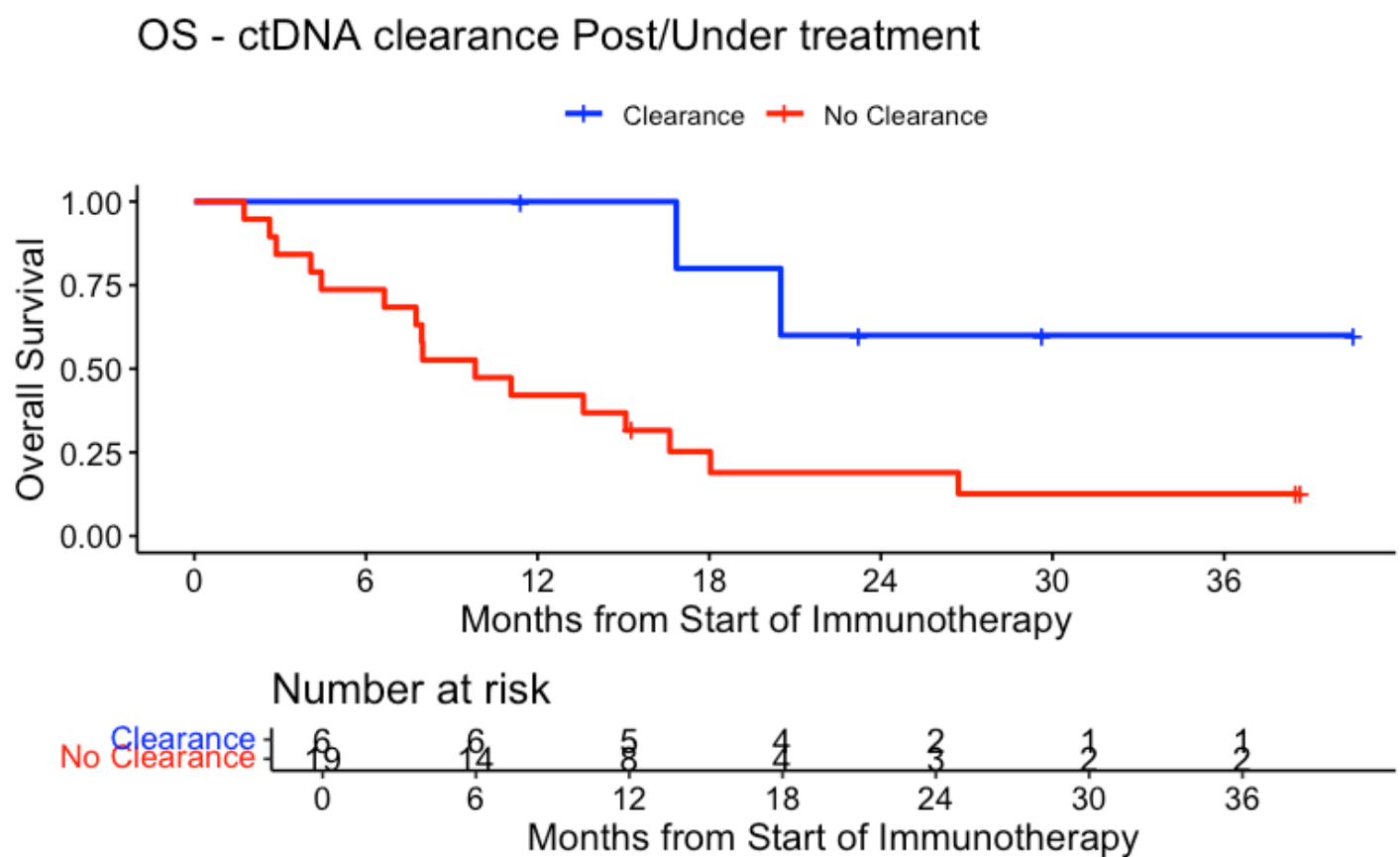
```
event_summary <- circ_data %>%
  group_by(ctDNA.Dynamics) %>%
  summarise(
    Total = n(),
    Events = sum(OS.Event),
    Fraction = Events / n(),
    Percentage = (Events / n()) * 100
  )
print(event_summary)
```

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.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=6, palette=c("blue","red"), title="OS - ctDNA clearance Post/Under treatment", ylab= "Overall Survival", xlab="Months from Start of Immunotherapy", legend.labs =c("Clearance", "No Clearance"), legend.title="")
```

[Hide](#)

```
summary(KM_curve, times= c(0, 12, 24, 36))
```

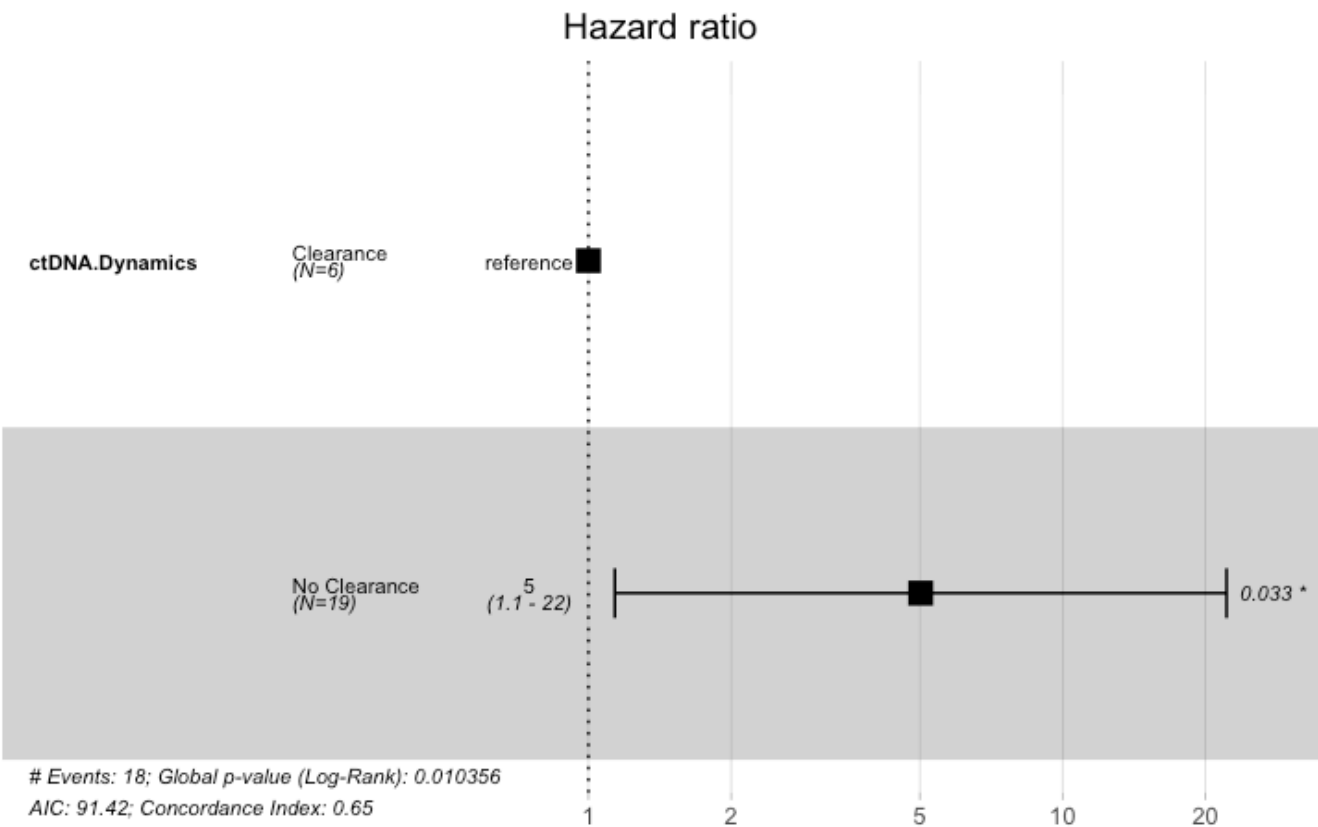
```
Call: survfit(formula = surv_object ~ ctDNA.Dynamics, data = circ_data,
  conf.int = 0.95, conf.type = "log-log")
```

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)
```



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_CI, 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)
```

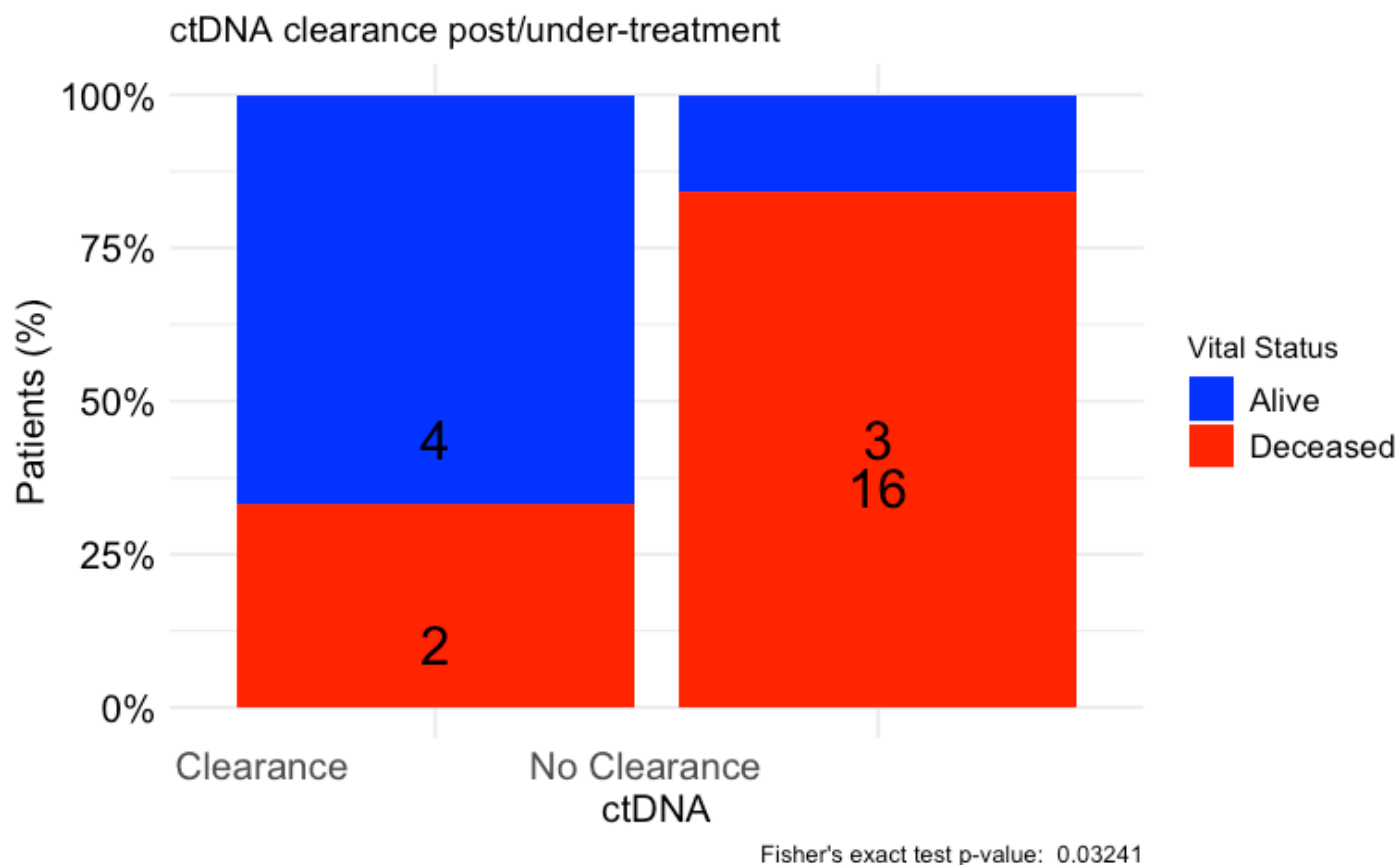
	Alive	Deceased
Clearance	4	2
No Clearance	3	16

[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 = "black", 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 size
        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

```



#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 #first we create the variable for the ctDNA & NAC combina
tion, and we assign values
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

Hide

```
print(chi_square_test)
```

Pearson's Chi-squared test

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

Hide

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

Fisher's Exact Test for Count Data

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

Hide

```
print(contingency_table)
```

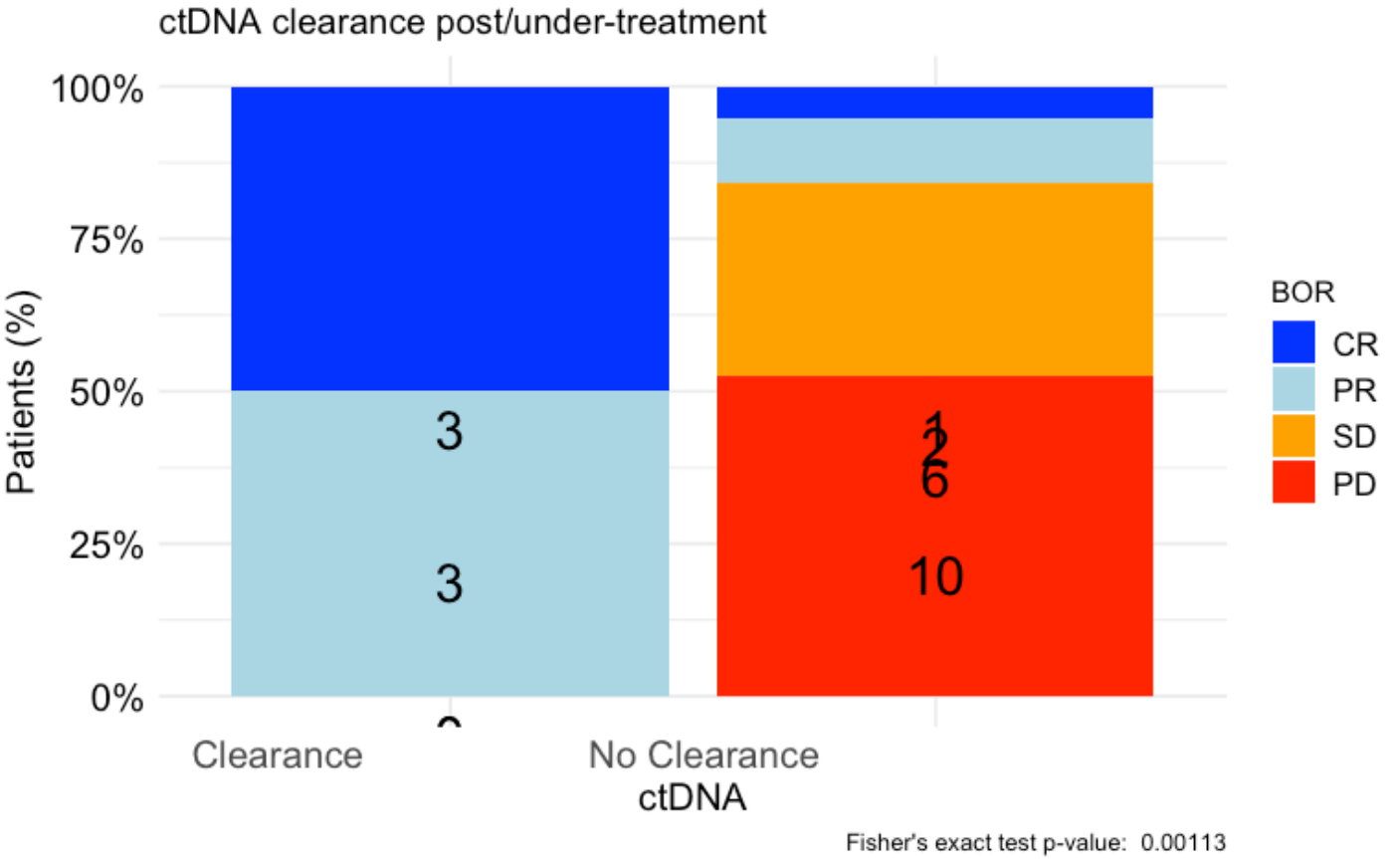
	CR	PR	SD	PD
Clearance	3	3	0	0
No Clearance	1	2	6	10

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 = "black", 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 size
        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

```



#Multivariate cox regression for PFS

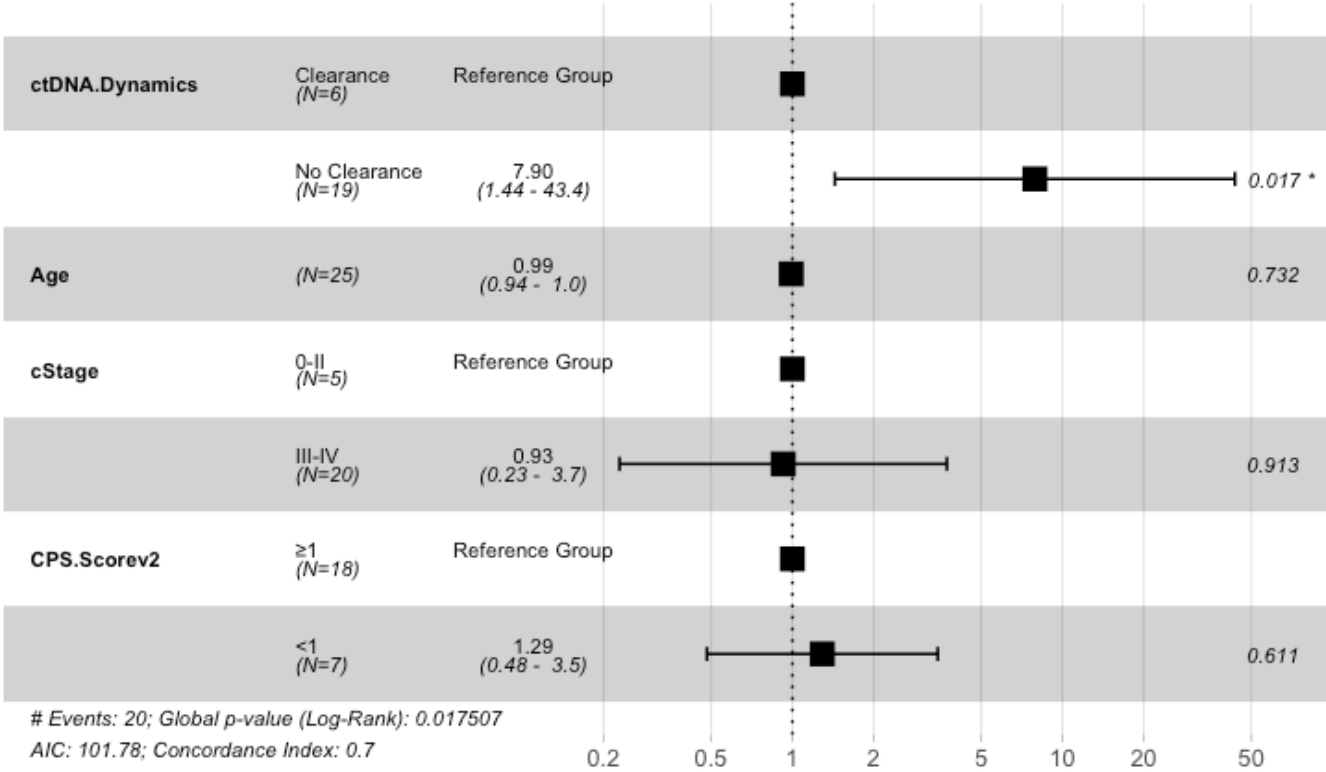
Hide

```
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 #first we create the variable for the ctDNA & NAC combina
tion, and we assign values
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$cStage <- factor(circ_data$cStage, levels = c("0-II", "III-IV"))
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 + cStage + CPS.Scorev2, data=circ_da
ta)
ggforest(cox_fit, data = circ_data, main = "Multivariate Regression Model for PFS", refL
abel = "Reference Group")
```

Multivariate Regression Model for PFS



Hide

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

#Multivariate cox regression for PFS v2

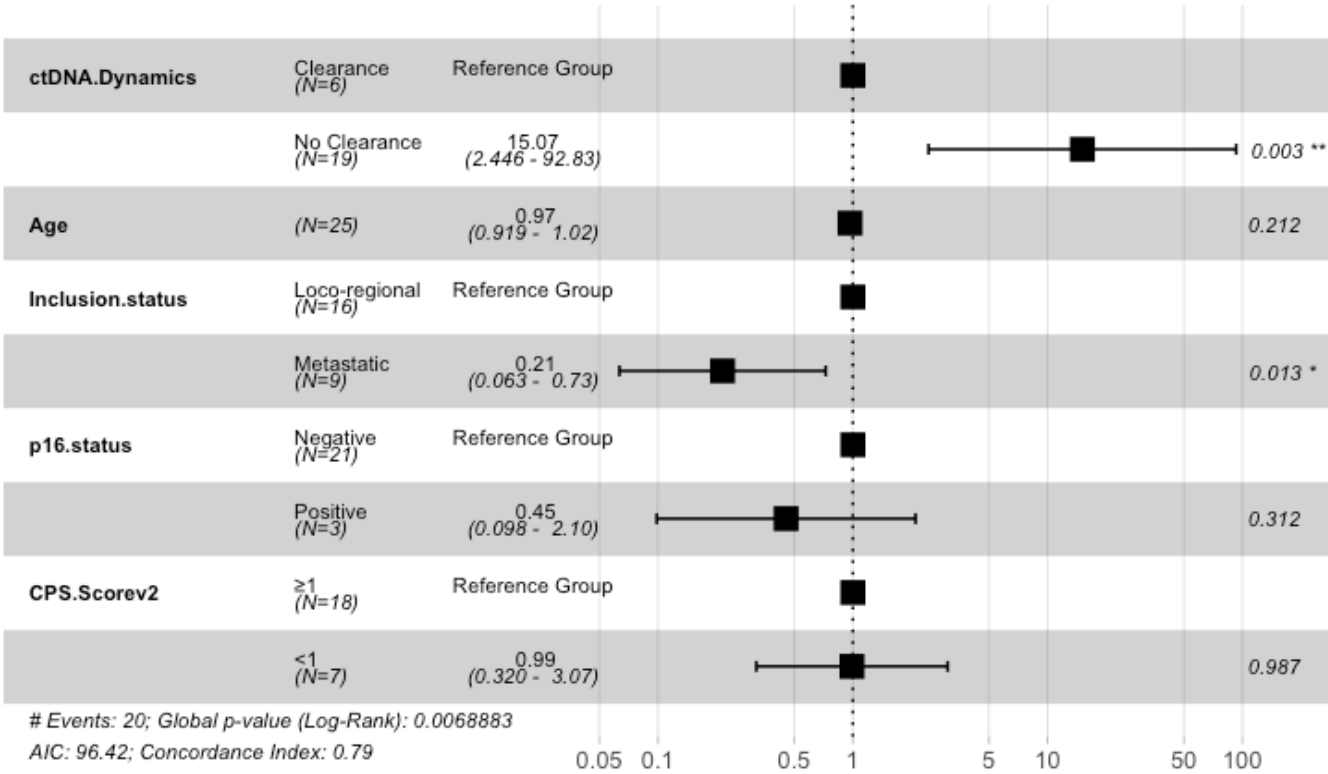
Hide

```
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 #first we create the variable for the ctDNA & NAC combina
tion, and we assign values
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



Hide

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

#Multivariate cox regression for PFS v3

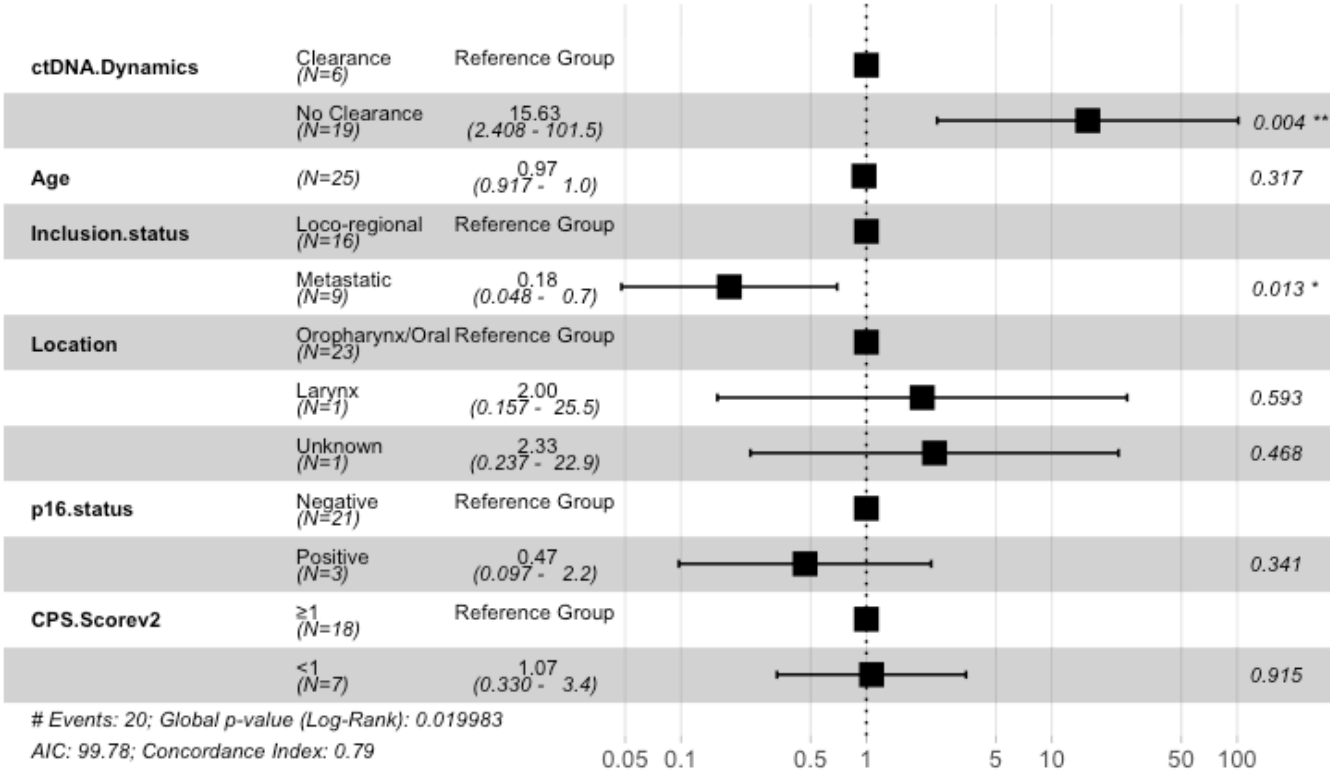
Hide

```
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 #first we create the variable for the ctDNA & NAC combina
tion, and we assign values
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$Location <- factor(circ_data$Location, levels = c("Oropharynx/Oral", "Larynx",
"Unknown"))
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 + Location + p16.
status + CPS.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



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_CI, 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_CI, 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$PFS.Event)
circ_data$cStage <- factor(circ_data$cStage, levels = c("0-II", "III-IV")) #univariate for Stage
cox_fit <- coxph(surv_object ~ cStage, data=circ_data)
summary(cox_fit)
```

Call:

```
coxph(formula = surv_object ~ cStage, data = circ_data)
```

n= 25, number of events= 20

	coef	exp(coef)	se(coef)	z	Pr(> z)
cStageIII-IV	0.9241	2.5196	0.6372	1.45	0.147

	exp(coef)	exp(-coef)	lower .95	upper .95
cStageIII-IV	2.52	0.3969	0.7226	8.785

Concordance= 0.585 (se = 0.047)

Likelihood ratio test= 2.55 on 1 df, p=0.1

Wald test = 2.1 on 1 df, p=0.1

Score (logrank) test = 2.24 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_CI, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = 2.52 (0.72-8.78); p = 0.147"
```

[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-regional", "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](#)

```
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 = 0.89 (0.36-2.2); p = 0.809"
```

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$PFS.Event)
circ_data$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_CI, 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$PFS.Event)
circ_data$Location <- factor(circ_data$Location, levels = c("Oropharynx/Oral", "Larynx", "Unknown")) #univariate for disease location
cox_fit <- coxph(surv_object ~ p16.status, data=circ_data)
```

Warning: Loglik converged before variable 2 ; coefficient may be infinite.

[Hide](#)

```
summary(cox_fit)
```

Call:

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

n= 25, number of events= 20

	coef	exp(coef)	se(coef)	z	Pr(> z)
p16.statusPositive	2.431e-01	1.275e+00	6.402e-01	0.380	0.704
p16.statusUnknown	-1.815e+01	1.309e-08	6.641e+03	-0.003	0.998

	exp(coef)	exp(-coef)	lower .95	upper .95
p16.statusPositive	1.275e+00	7.842e-01	0.3636	4.472
p16.statusUnknown	1.309e-08	7.641e+07	0.0000	Inf

Concordance= 0.526 (se = 0.041)

Likelihood ratio test= 3.49 on 2 df, p=0.2

Wald test = 0.14 on 2 df, p=0.9

Score (logrank) test = 1.92 on 2 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_CI, 2), "); p = ", round(p_value, 3))
print(label_text)
```

```
[1] "HR = -18.15 (0.78-76405588.46); p = 0.64"
```

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")) #univariate 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_CI, 2), "); p = ", round(p_value, 3))
print(label_text)

```

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

#Multivariate cox regression for OS

[Hide](#)

```

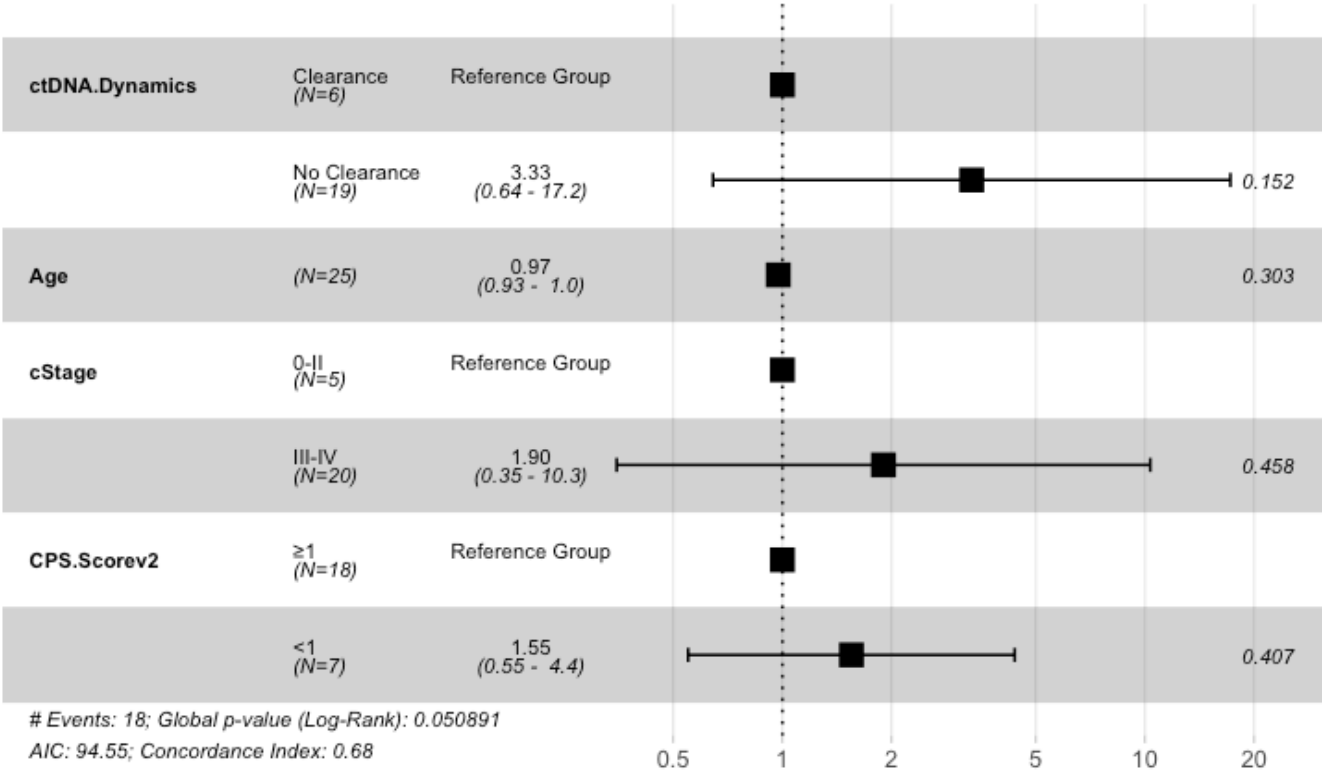
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("EORTC HNSCC ICI Clinical Data.csv")
circ_data <- circ_data[circ_data$ctDNA.available=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Base!="",]
circ_data <- circ_data[circ_data$ctDNA.Base=="POSITIVE",]
circ_datadf <- as.data.frame(circ_data)

circ_data$ctDNA.Dynamics <- NA #first we create the variable for the ctDNA & NAC combination, and we assign values
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$cStage <- factor(circ_data$cStage, levels = c("0-II", "III-IV"))
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 + cStage + CPS.Scorev2, data=circ_data)
ggforest(cox_fit, data = circ_data, main = "Multivariate Regression Model for OS", refLabel = "Reference Group")

```

Multivariate Regression Model for OS



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

#Multivariate cox regression for OS v2

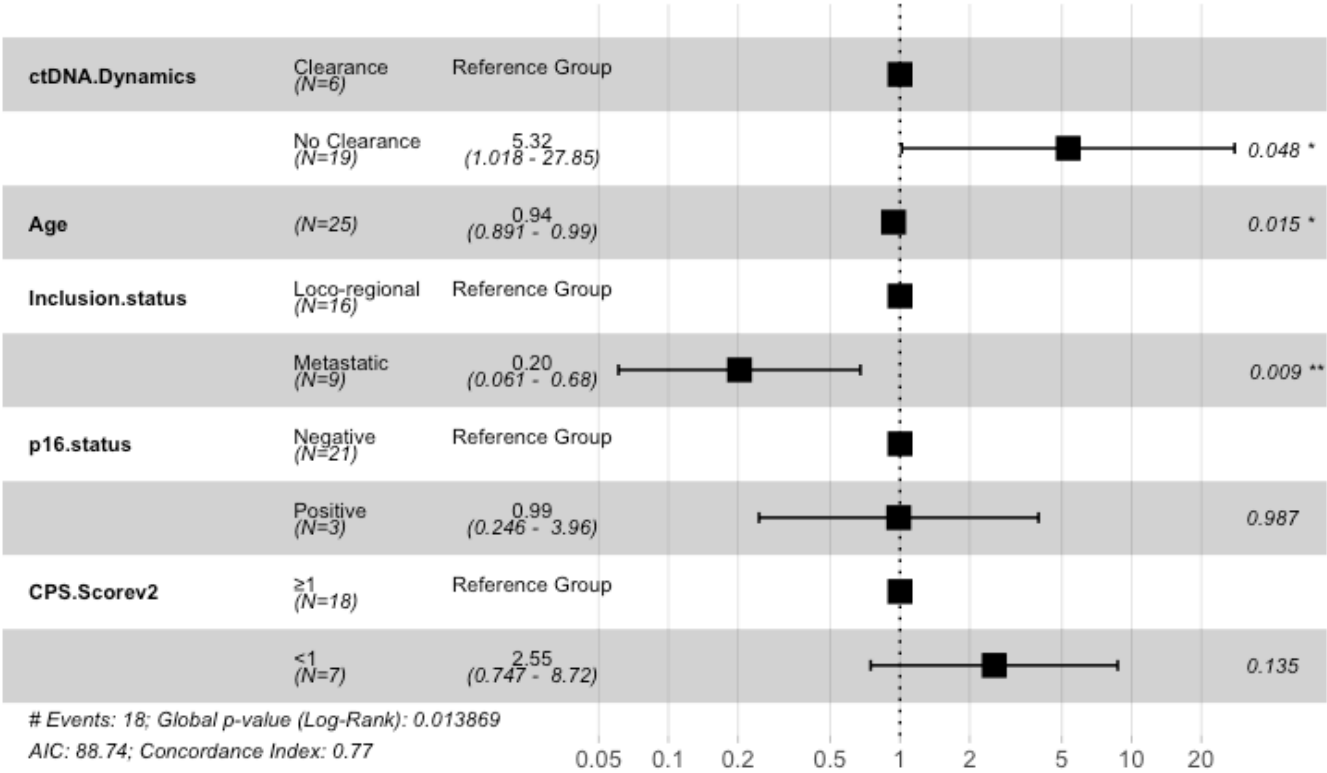
Hide

```
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 #first we create the variable for the ctDNA & NAC combina
tion, and we assign values
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



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