Code

# Ayanambakkam et al_ctDNA in UTUC Clinical Analysis

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

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```
#ctDNA at MRD by Stage
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Clinical Data 102025.csv")
circ_data <- circ_data[circ_data$Final.cohort=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.MRD!="",]
circ_data$ctDNA.MRD <- factor(circ_data$ctDNA.MRD, levels=c("NEGATIVE","POSITIVE"))
circ_data <- subset(circ_data, ctDNA.MRD %in% c("NEGATIVE", "POSITIVE"))
circ_data$Stage <- factor(circ_data$Stage, levels=c("0/I","II","III"))
positive_counts_by_stage <- aggregate(circ_data$ctDNA.MRD == "POSITIVE", by=list(circ_data$Stage), FUN=sum)
total_counts_by_stage <- aggregate(circ_data$ctDNA.MRD, by=list(circ_data$Stage), FUN=length)
combined_data <- data.frame(
  Stage = total_counts_by_stage$Group.1,
  Total_Count = total_counts_by_stage$x,
  Positive_Count = positive_counts_by_stage$x,
  Rate = (positive_counts_by_stage$x / total_counts_by_stage$x) * 100  # Convert to percentage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$ctDNA.MRD == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Convert to percentage
overall_row <- data.frame(
  Stage = "Overall",
  Total_Count = overall_total_count,
  Positive_Count = overall_positive_count,
  Rate = sprintf("%.2f%%", overall_positivity_rate)
)
combined_data <- rbind(combined_data, overall_row)
print(combined_data)
```

```
    Stage Total_Count Positive_Count   Rate
1     0/I           6              2 33.33%
2      II           6              4 66.67%
3     III          16              9 56.25%
4 Overall          28             15 53.57%
```

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```
#ctDNA at MRD by NAC status
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Clinical Data 102025.csv")
circ_data <- circ_data[circ_data$Final.cohort=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.MRD!="",]
circ_data$ctDNA.MRD <- factor(circ_data$ctDNA.MRD, levels=c("NEGATIVE","POSITIVE"))
circ_data <- subset(circ_data, ctDNA.MRD %in% c("NEGATIVE", "POSITIVE"))
circ_data$NAC <- factor(circ_data$NAC, levels=c("FALSE","TRUE"))
positive_counts_by_stage <- aggregate(circ_data$ctDNA.MRD == "POSITIVE", by=list(circ_data$NAC), FUN=sum)
total_counts_by_stage <- aggregate(circ_data$ctDNA.MRD, by=list(circ_data$NAC), FUN=length)
combined_data <- data.frame(
  Stage = total_counts_by_stage$Group.1,
  Total_Count = total_counts_by_stage$x,
  Positive_Count = positive_counts_by_stage$x,
  Rate = (positive_counts_by_stage$x / total_counts_by_stage$x) * 100  # Convert to percentage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$ctDNA.MRD == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Convert to percentage
overall_row <- data.frame(
  Stage = "Overall",
  Total_Count = overall_total_count,
  Positive_Count = overall_positive_count,
  Rate = sprintf("%.2f%%", overall_positivity_rate)
)
combined_data <- rbind(combined_data, overall_row)
print(combined_data)
```

```
    Stage Total_Count Positive_Count   Rate
1   FALSE          23             12 52.17%
2    TRUE           5              3 60.00%
3 Overall          28             15 53.57%
```

Hide

```
#ctDNA at Surveillance by Stage
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Clinical Data 102025.csv")
circ_data <- circ_data[circ_data$Final.cohort=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Surveillance!="",]
circ_data$ctDNA.Surveillance <- factor(circ_data$ctDNA.Surveillance, levels=c("NEGATIVE","POSITIVE"))
circ_data$Stage <- factor(circ_data$Stage, levels=c("0/I","II","III"))
positive_counts_by_stage <- aggregate(circ_data$ctDNA.Surveillance == "POSITIVE", by=list(circ_data$Stage), FUN=s
um)
total_counts_by_stage <- aggregate(circ_data$ctDNA.Surveillance, by=list(circ_data$Stage), FUN=length)
combined_data <- data.frame(
  Stage = total_counts_by_stage$Group.1,
  Total_Count = total_counts_by_stage$x,
  Positive_Count = positive_counts_by_stage$x,
  Rate = (positive_counts_by_stage$x / total_counts_by_stage$x) * 100  # Convert to percentage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$ctDNA.Surveillance == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Convert to percentage
overall_row <- data.frame(
  Stage = "Overall",
  Total_Count = overall_total_count,
  Positive_Count = overall_positive_count,
  Rate = sprintf("%.2f%%", overall_positivity_rate)
)
combined_data <- rbind(combined_data, overall_row)
print(combined_data)
```

```
    Stage Total_Count Positive_Count   Rate
1     0/I           9              3 33.33%
2      II           9              7 77.78%
3     III          22             12 54.55%
4 Overall          40             22 55.00%
```

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```
#ctDNA at anytime by Stage
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Clinical Data 102025.csv")
circ_data <- circ_data[circ_data$Final.cohort=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.anytime!="",]
circ_data$ctDNA.anytime <- factor(circ_data$ctDNA.anytime, levels=c("NEGATIVE","POSITIVE"))
circ_data$Stage <- factor(circ_data$Stage, levels=c("0/I","II","III"))
positive_counts_by_stage <- aggregate(circ_data$ctDNA.anytime == "POSITIVE", by=list(circ_data$Stage), FUN=sum)
total_counts_by_stage <- aggregate(circ_data$ctDNA.anytime, by=list(circ_data$Stage), FUN=length)
combined_data <- data.frame(
  Stage = total_counts_by_stage$Group.1,
  Total_Count = total_counts_by_stage$x,
  Positive_Count = positive_counts_by_stage$x,
  Rate = (positive_counts_by_stage$x / total_counts_by_stage$x) * 100  # Convert to percentage
)
combined_data$Rate <- sprintf("%.2f%%", combined_data$Rate)
overall_total_count <- nrow(circ_data)
overall_positive_count <- nrow(circ_data[circ_data$ctDNA.anytime == "POSITIVE",])
overall_positivity_rate <- (overall_positive_count / overall_total_count) * 100  # Convert to percentage
overall_row <- data.frame(
  Stage = "Overall",
  Total_Count = overall_total_count,
  Positive_Count = overall_positive_count,
  Rate = sprintf("%.2f%%", overall_positivity_rate)
)
combined_data <- rbind(combined_data, overall_row)
print(combined_data)
```

```
    Stage Total_Count Positive_Count   Rate
1     0/I           9              3 33.33%
2      II          10              8 80.00%
3     III          28             22 78.57%
4 Overall          47             33 70.21%
```

#Demographics Table

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```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Clinical Data 102025.csv")
circ_data <- circ_data[circ_data$Final.cohort=="TRUE",]

circ_data_subset <- circ_data %>%
  select(
    Age,
    Sex,
    Stage,
    NAC,
    ACT,
    ACT.type,
    RFS.Event,
    OS.Event,
    OS.months) %>%
  mutate(
    Age = as.numeric(Age),
    Sex = factor(Sex),
    Stage = factor(Stage),
    NAC = factor(NAC),
    ACT = factor(ACT),
    ACT.type = factor(ACT.type),
    RFS.Event = factor(RFS.Event, levels = c("FALSE", "TRUE"), labels = c("No Recurrence", "Recurrence")),
    OS.Event = factor(OS.Event, levels = c("FALSE", "TRUE"), labels = c("Alive", "Deceased")),
    OS.months = as.numeric(OS.months))
table1 <- circ_data_subset %>%
  tbl_summary(
    statistic = list(
      all_continuous() ~ "{median} ({min} - {max})",
      all_categorical() ~ "{n} ({p}%)")) %>%
  bold_labels()
table1
```

| **Characteristic** | **N = 47**[1] |
|---|---|
| **Age** | 77 (47 - 92) |
| **Sex** | |
| Female | 17 (36%) |
| Male | 30 (64%) |
| **Stage** | |
| 0/I | 9 (19%) |
| II | 10 (21%) |
| III | 28 (60%) |
| **NAC** | |
| FALSE | 40 (85%) |
| TRUE | 7 (15%) |
| **ACT** | |
| FALSE | 13 (28%) |
| TRUE | 34 (72%) |
| **ACT.type** | |
| | 13 (28%) |
| Chemotherapy | 18 (38%) |
| Immunotherapy | 16 (34%) |
| **RFS.Event** | |
| No Recurrence | 23 (49%) |
| Recurrence | 24 (51%) |
| **OS.Event** | |
| Alive | 41 (87%) |
| Deceased | 6 (13%) |
| **OS.months** | 21 (3 - 48) |

[1] Median (Min - Max); n (%)

Hide

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

| **Characteristic** | **N = 47**[1] |
|---|---|
| **Age** | 77 (47 - 92) |
| **Sex** | |
| Female | 17 (36%) |
| Male | 30 (64%) |
| **Stage** | |
| 0/I | 9 (19%) |
| II | 10 (21%) |
| III | 28 (60%) |
| **NAC** | |
| FALSE | 40 (85%) |

[1]Median (Min - Max); n (%)

| Characteristic | N = 47[1] |
|---|---|
| TRUE | 7 (15%) |
| **ACT** | |
| FALSE | 13 (28%) |
| TRUE | 34 (72%) |
| **ACT.type** | |
| | 13 (28%) |
| Chemotherapy | 18 (38%) |
| Immunotherapy | 16 (34%) |
| **RFS.Event** | |
| No Recurrence | 23 (49%) |
| Recurrence | 24 (51%) |
| **OS.Event** | |
| Alive | 41 (87%) |
| Deceased | 6 (13%) |
| **OS.months** | 21 (3 - 48) |

[1]Median (Min - Max); n (%)

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```
save_as_docx(fit1, path= "~/Downloads/1. Demographics Table_RWE UTUC.docx")
```

#Overview plot

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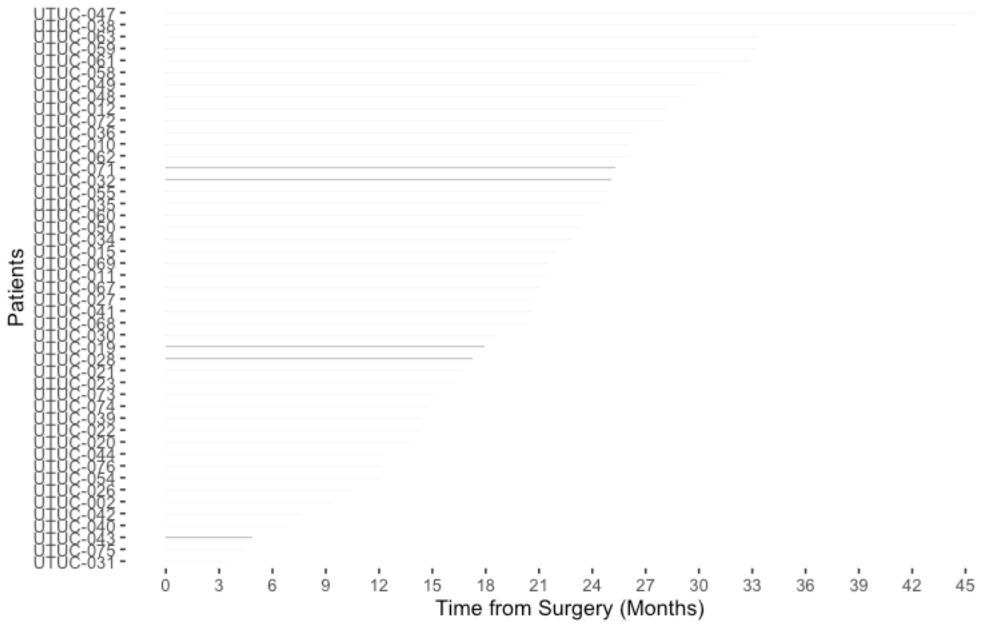
```
rm(list=ls())
setwd("~/Downloads")
clinstage<- read.csv("RWE UTUC_OP 102025.csv")
clinstage <- clinstage[clinstage$Final.cohort=="TRUE",]
clinstage_df<- as.data.frame(clinstage)

#Display the swimmer plot with the label box
oplot<-swimmer_plot(df=clinstage_df,
                    id='PatientName',
                    end='fu.diff.months',
                    fill='gray',
                    width=.01,)
oplot <- oplot + theme(panel.border = element_blank())
oplot <- oplot + scale_y_continuous(breaks = seq(0, 96, by = 3))
oplot <- oplot + labs(x ="Patients" , y="Time from Surgery (Months)")
oplot
```



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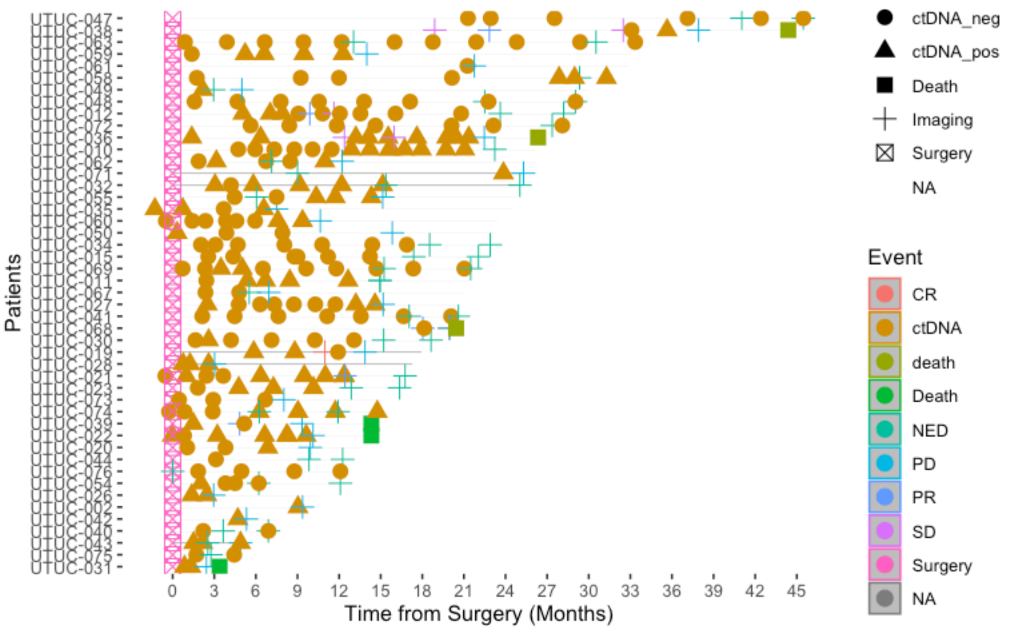
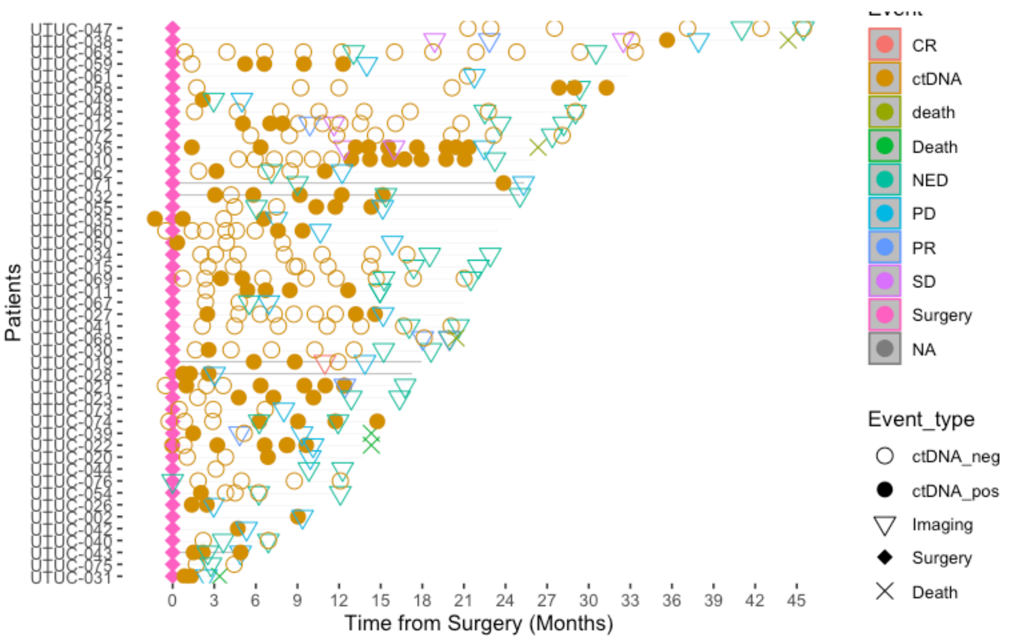
```
##plot events
oplot_ev1 <- oplot + swimmer_points(df_points=clinstage_df,
                                    id='PatientName',
                                    time='date.diff.months',
                                    name_shape ='Event_type',
                                    name_col = 'Event',
                                    size=3.5,fill='black',
                                    #col='darkgreen'
)
oplot_ev1
```



Hide

```
#Shape customization to Event_type

oplot_ev1.1 <- oplot_ev1 + ggplot2::scale_shape_manual(name="Event_type",values=c(1,16,6,18,4),breaks=c('ctDNA_ne
g','ctDNA_pos','Imaging','Surgery','Death'))
oplot_ev1.1
```



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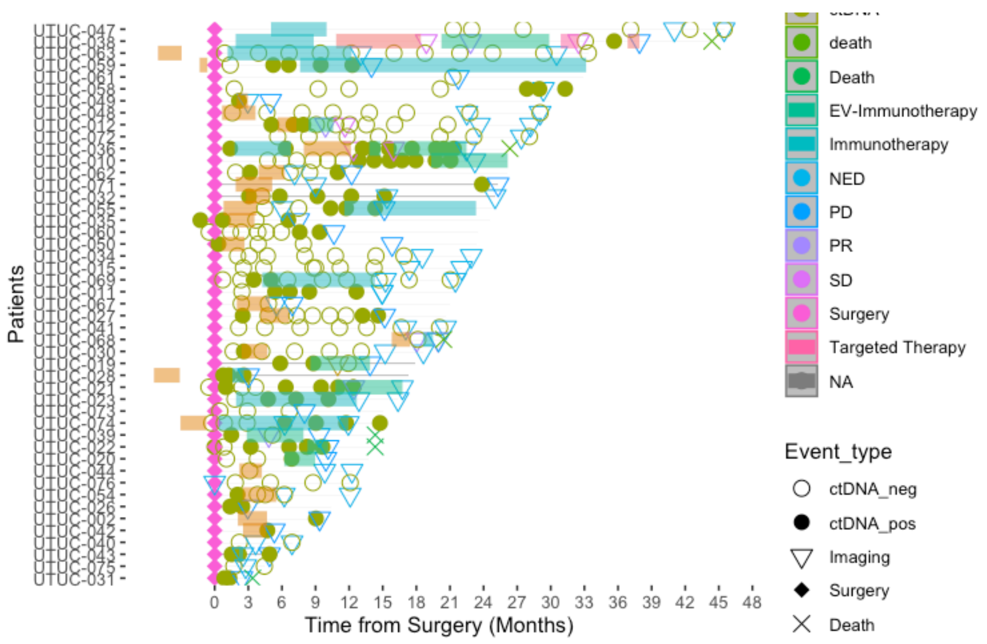
```
#plot treatment

oplot_ev2 <- oplot_ev1.1 + swimmer_lines(df_lines=clinstage_df,
                                         id='PatientName',
                                         start='Tx_start.months',
                                         end='Tx_end.months',
                                         name_col='Tx_type',
                                         size=3.5,
                                         name_alpha = 1.0)
oplot_ev2 <- oplot_ev2 + guides(linetype = guide_legend(override.aes = list(size = 5, color = "black")))
oplot_ev2
```



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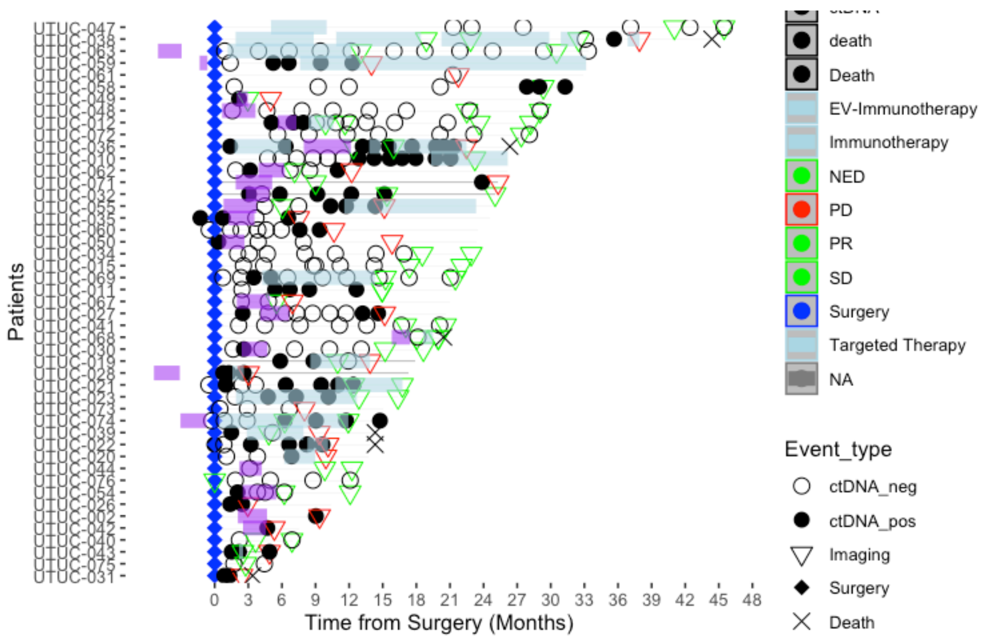
```
#colour customization
oplot_ev2.2 <- oplot_ev2 + ggplot2::scale_color_manual(name="Event",values=c( "lightblue","purple","green", "blac
k", "black", "black", "lightblue","lightblue","green","red","green","green","blue","lightblue", "blue", "blue"))
oplot_ev2.2
```



#RFS by ctDNA status at MRD Window

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Clinical Data 102025.csv")
circ_data <- circ_data[circ_data$Final.cohort=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.MRD!="",]
circ_data$RFS.MRD <- circ_data$RFS - circ_data$ctDNA.timing
circ_data$RFS.MRD.months <- circ_data$RFS.MRD / 30.437

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

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

                    n events median 0.95LCL 0.95UCL
ctDNA.MRD=NEGATIVE 13      3     NA   12.62      NA
ctDNA.MRD=POSITIVE 15     11   9.07    6.77      NA
```

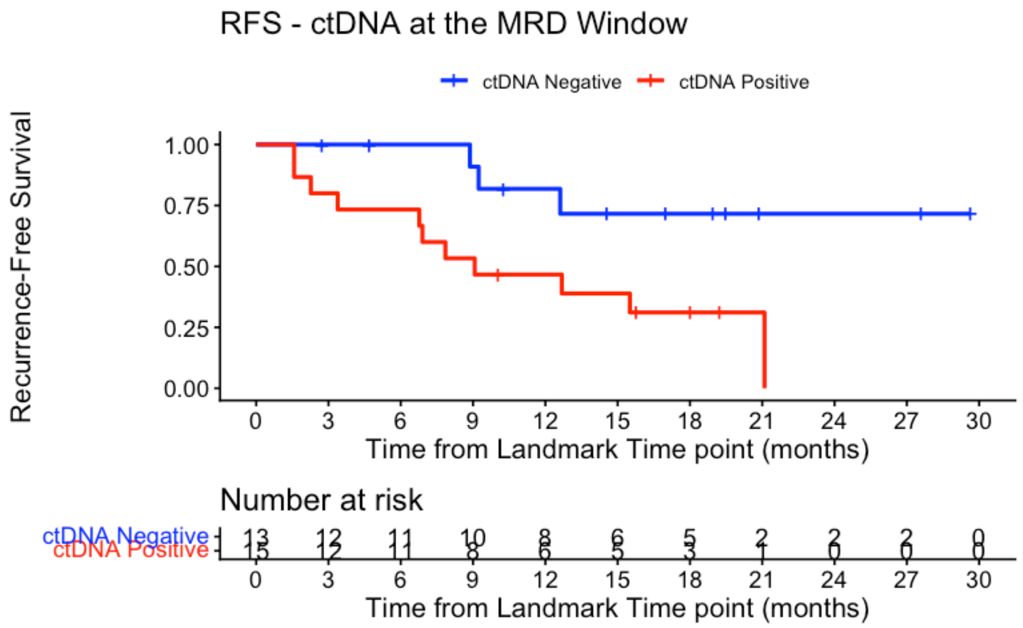
Hide

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

```
# A tibble: 2 × 5
  ctDNA.MRD Total Events Fraction Percentage
  <chr>     <int>  <int>    <dbl>      <dbl>
1 NEGATIVE     13      3    0.231       23.1
2 POSITIVE     15     11    0.733       73.3
```

Hide

```
surv_object <-Surv(time = circ_data$RFS.MRD.months, event = circ_data$RFS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.MRD, data = circ_data,conf.int=0.95,conf.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=3, palett
e=c("blue","red"), title="RFS - ctDNA at the MRD Window", ylab= "Recurrence-Free Survival", xlab="Time from Landm
ark Time point (months)", legend.labs=c("ctDNA Negative", "ctDNA Positive"), legend.title="")
```



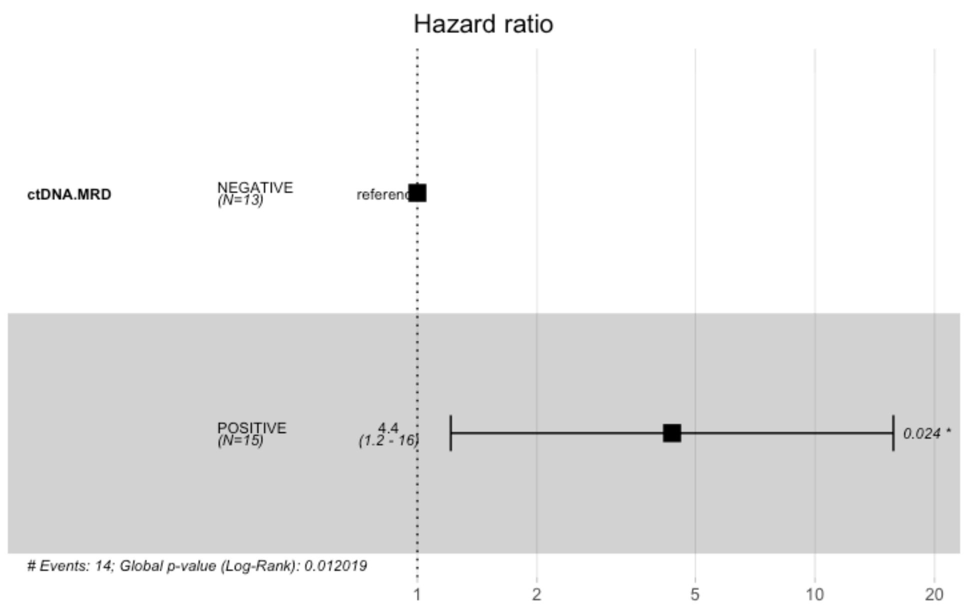
```
summary(KM_curve, times= c(0, 12, 18))
```

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

                ctDNA.MRD=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      8       2    0.818   0.116        0.447        0.951
   18      5       1    0.716   0.140        0.350        0.899

                ctDNA.MRD=POSITIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0     15       0    1.000   0.000        1.000        1.000
   12      6       8    0.467   0.129        0.212        0.687
   18      3       2    0.311   0.124        0.102        0.550
```

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



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```
summary(cox_fit)
```

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

  n= 28, number of events= 14

                    coef exp(coef) se(coef)     z Pr(>|z|)
ctDNA.MRDPOSITIVE 1.4752    4.3720   0.6539 2.256   0.0241 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

                  exp(coef) exp(-coef) lower .95 upper .95
ctDNA.MRDPOSITIVE     4.372     0.2287     1.214     15.75

Concordance= 0.677  (se = 0.057 )
Likelihood ratio test= 6.31  on 1 df,   p=0.01
Wald test            = 5.09  on 1 df,   p=0.02
Score (logrank) test = 6.05  on 1 df,   p=0.01
```

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 = 4.37 (1.21-15.75); p = 0.024"
```

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```
circ_data$ctDNA.MRD <- factor(circ_data$ctDNA.MRD, levels = c("NEGATIVE", "POSITIVE"), labels = c("Negative", "Po
sitive"))
circ_data$RFS.Event <- factor(circ_data$RFS.Event, levels = c("FALSE", "TRUE"), labels = c("No Recurrence", "Recu
rrence"))
contingency_table <- table(circ_data$ctDNA.MRD, circ_data$RFS.Event)
chi_square_test <- chisq.test(contingency_table)
print(chi_square_test)
```

```

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

data:  contingency_table
X-squared = 5.1692, df = 1, p-value = 0.02299
```

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

```

	Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.0213
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  1.28780 74.44434
sample estimates:
odds ratio
  8.328277
```

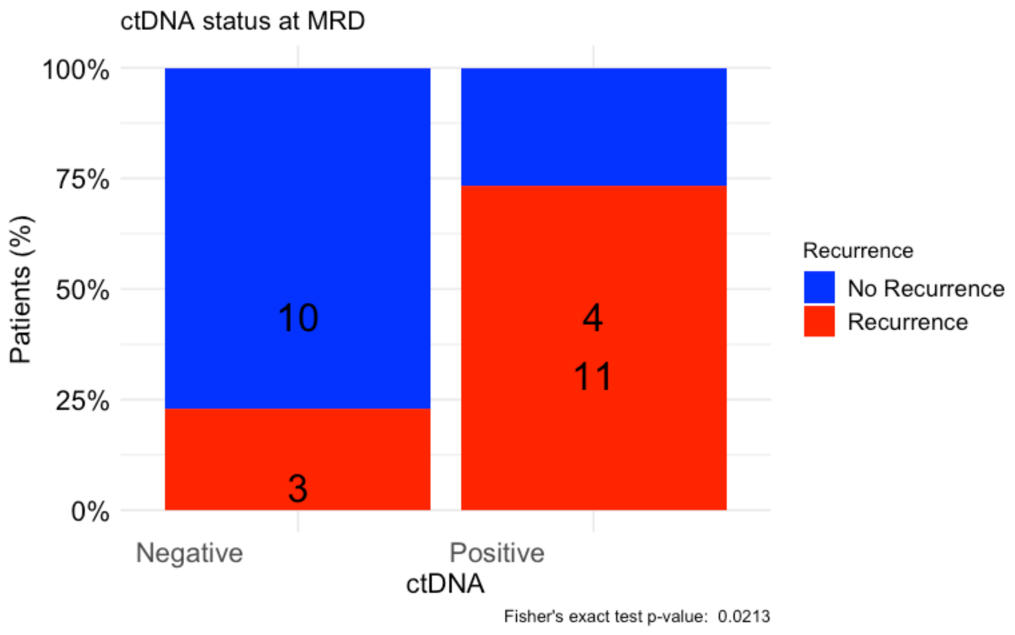
Hide

```
print(contingency_table)
```

```
          No Recurrence Recurrence
 Negative            10          3
 Positive             4         11
```

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 at MRD",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "Recurrence",
       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 Recurrence" = "blue", "Recurrence" = "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 Recurrence label size
```



#RFS by ctDNA status at Surveillance Window

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```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Clinical Data 102025.csv")
circ_data <- circ_data[circ_data$Final.cohort=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Surveillance!="",]

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

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

                             n events median 0.95LCL 0.95UCL
ctDNA.Surveillance=NEGATIVE 18      3     NA      NA      NA
ctDNA.Surveillance=POSITIVE 22     17   13.9    10.1      NA
```

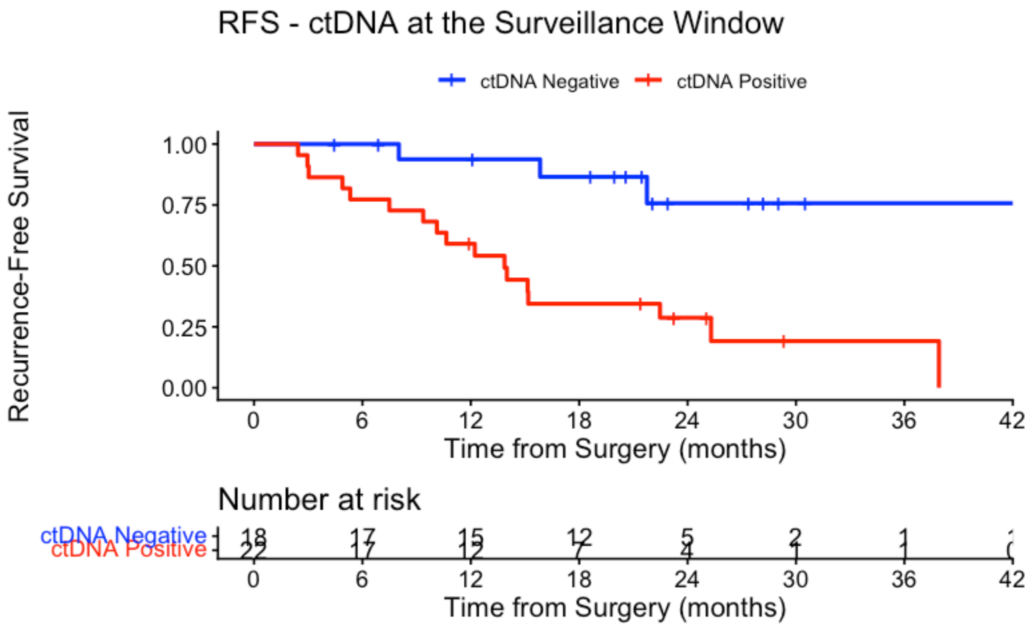
Hide

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

```
# A tibble: 2 × 5
  ctDNA.Surveillance Total Events Fraction Percentage
  <chr>              <int>  <int>    <dbl>      <dbl>
1 NEGATIVE              18      3    0.167       16.7
2 POSITIVE              22     17    0.773       77.3
```

Hide

```
surv_object <-Surv(time = circ_data$RFS.months, event = circ_data$RFS.Event)
KM_curve <- survfit(surv_object ~ ctDNA.Surveillance, data = circ_data,conf.int=0.95,conf.type="log-log")
ggsurvplot(KM_curve, data = circ_data, pval = FALSE, conf.int = FALSE, risk.table = TRUE, break.time.by=6, palett
e=c("blue","red"), title="RFS - ctDNA at the Surveillance Window", ylab= "Recurrence-Free Survival", xlab="Time f
rom Surgery (months)", legend.labs=c("ctDNA Negative", "ctDNA Positive"), legend.title="")
```



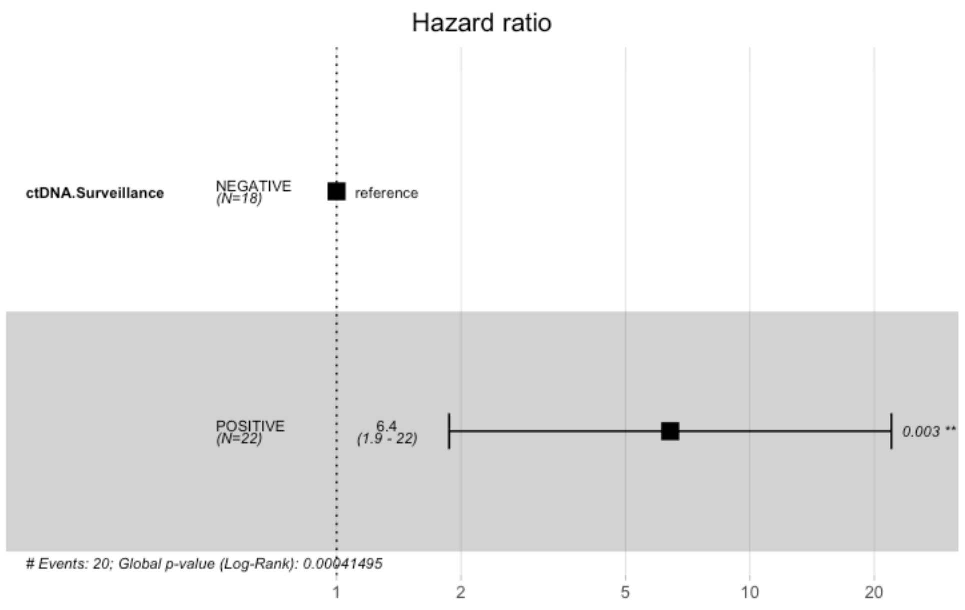
Hide

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

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

                ctDNA.Surveillance=NEGATIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0     18       0    1.000  0.0000        1.000        1.000
   12     15       1    0.938  0.0605        0.632        0.991
   24      5       2    0.757  0.1277        0.401        0.919

                ctDNA.Surveillance=POSITIVE
 time n.risk n.event survival std.err lower 95% CI upper 95% CI
    0     22       0    1.000   0.000        1.000        1.000
   12     12       9    0.591   0.105        0.361        0.762
   24      4       6    0.287   0.101        0.114        0.488
```

Hide

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



Hide

```
summary(cox_fit)
```

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

  n= 40, number of events= 20

                             coef exp(coef) se(coef)     z Pr(>|z|)
ctDNA.SurveillancePOSITIVE 1.8586    6.4151   0.6287 2.956  0.00311 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

                           exp(coef) exp(-coef) lower .95 upper .95
ctDNA.SurveillancePOSITIVE     6.415     0.1559     1.871     21.99

Concordance= 0.699  (se = 0.047 )
Likelihood ratio test= 12.46  on 1 df,   p=4e-04
Wald test            = 8.74  on 1 df,   p=0.003
Score (logrank) test = 11.47  on 1 df,   p=7e-04
```

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 = 6.42 (1.87-21.99); p = 0.003"
```

Hide

```
circ_data$ctDNA.Surveillance <- factor(circ_data$ctDNA.Surveillance, levels = c("NEGATIVE", "POSITIVE"), labels =
c("Negative", "Positive"))
circ_data$RFS.Event <- factor(circ_data$RFS.Event, levels = c("FALSE", "TRUE"), labels = c("No Recurrence", "Recu
rrence"))
contingency_table <- table(circ_data$ctDNA.Surveillance, circ_data$RFS.Event)
chi_square_test <- chisq.test(contingency_table)
print(chi_square_test)
```

```

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

data:  contingency_table
X-squared = 12.222, df = 1, p-value = 0.0004722
```

Hide

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

```

    Fisher's Exact Test for Count Data

data:  contingency_table
p-value = 0.0003284
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
   2.867735 118.989345
sample estimates:
odds ratio
  15.45796
```

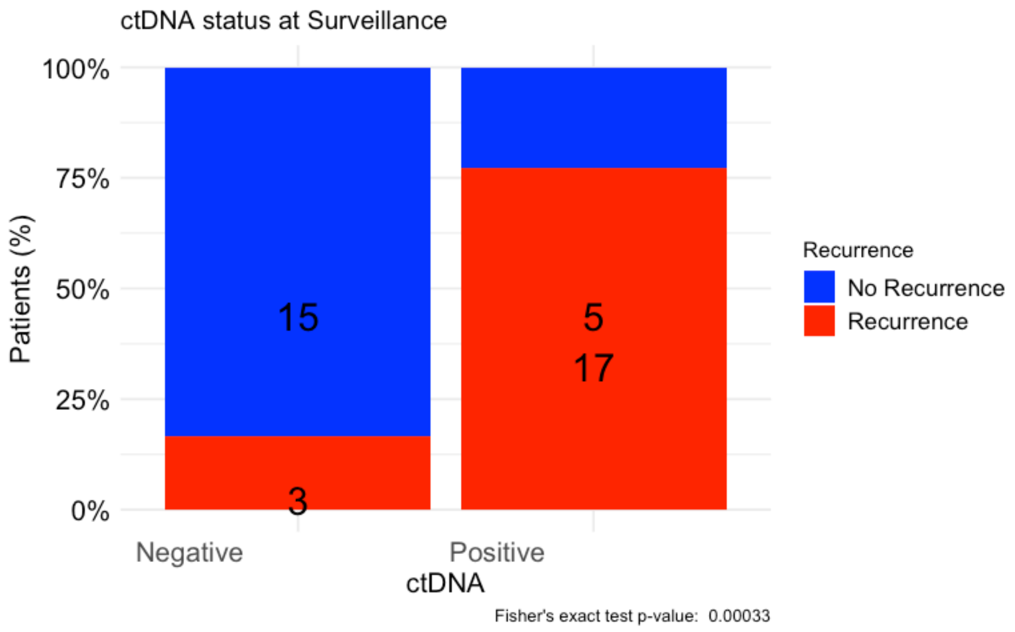
Hide

```
print(contingency_table)
```

```

           No Recurrence Recurrence
  Negative            15          3
  Positive             5         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 at Surveillance",
       x = "ctDNA",
       y = "Patients (%)",
       fill = "Recurrence",
       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 Recurrence" = "blue", "Recurrence" = "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 Recurrence label size
```



#Time-dependent analysis for RFS in longitudinal time points

Hide

```
rm(list=ls())
setwd("~/Downloads")
dt <- read_xlsx("CLIA UTUC Clinical Data_Time dependent.xlsx") |>
  clean_names() |>
  mutate(across(
    .cols = c(window_start_date, dfs_date, surveillance_1_date:surveillance_12_date),
    .fns = ~ as_date(as.Date(.x, format = "%Y-%m-%d"))
  )) |>
  filter(final_cohort == TRUE)

dt_biomarker <- dt |>
  select(pts_id, ct_dna_surveillance_available,
         window_start_date,
         surveillance_1_status:surveillance_12_date) |>
  filter(ct_dna_surveillance_available) |>
  pivot_longer(cols = surveillance_1_status:surveillance_12_date,
               names_to = c("visit_number", ".value"),
               names_pattern = "surveillance_(.)_(.*)") |>
  mutate(biomarker_time = day(days(date - window_start_date))) |>
  select(pts_id, biomarker_time, biomarker_status = status) |>
  filter(!is.na(biomarker_time))

glimpse(dt_biomarker)
```

```
Rows: 137
Columns: 3
$ pts_id           <chr> "UTUC-002", "UTUC-010", "UTUC-010", "UTUC-010", "UTUC-010", "UTUC-010", "UTUC-010", "UTU
C-010", "UTUC-010", "UTUC-010", "UTUC-011", "UTUC-011", "UTUC-…
$ biomarker_time   <dbl> 133, 144, 181, 223, 267, 307, 349, 392, 433, 475, 74, 164, 204, 257, 385, 107, 145, 190,
267, 411, 78, 133, 267, 274, 341, 433, 42, 75, 21, 64, 111, 1…
$ biomarker_status <chr> "POSITIVE", "NEGATIVE", "NEGATIVE", "NEGATIVE", "NEGATIVE", "NEGATIVE", "NEGATIVE", "POS
ITIVE", "POSITIVE", "POSITIVE", "NEGATIVE", "POSITIVE", "POSIT…
```

Hide

```
dt_survival <- dt |>
  select(pts_id, ct_dna_surveillance_available,
         window_start_date:dfs_date, dfs_event) |>  # Added dfs_event here
  filter(ct_dna_surveillance_available) |>
  mutate(dfs_time = (dfs_date - window_start_date),
         dfs_time = day(days(dfs_time)),
         dfs_event = as.numeric(dfs_event)) |>
  select(pts_id, dfs_time, dfs_event)

glimpse(dt_survival)
```

```
Rows: 38
Columns: 3
$ pts_id    <chr> "UTUC-002", "UTUC-010", "UTUC-011", "UTUC-012", "UTUC-015", "UTUC-026", "UTUC-027", "UTUC-028",
"UTUC-030", "UTUC-031", "UTUC-032", "UTUC-034", "UTUC-035", "…
$ dfs_time  <dbl> 143, 707, 651, 636, 671, 90, 260, 13, 424, 74, 614, 697, 120, 314, 594, 210, 627, 23, 72, 1080,
773, 482, 201, 347, 893, 426, 324, 662, 182, 557, 77, 219, 61…
$ dfs_event <dbl> 1, 0, 0, 0, 0, 1, 1, 1, 0, 1, 0, 0, 1, 1, 1, 0, 0, 1, 1, 0, 0, 1, 0, 1, 0, 1, 1, 1, 1, 0, 0, 0,
1, 0, 1, 0, 0, 0
```

Hide

```
aux <- dt_survival %>%
  filter(dfs_time <= 0)

tab <- left_join(aux, dt) |>
  select(pts_id, window_start_date, dfs_time, dfs_date,
         surveillance_1_date:surveillance_12_date) |>
  mutate(across(.cols = dfs_date:surveillance_12_date,
                .fns = ~ as_date(.x))) |>
  select(pts_id, window_start_date, dfs_date, dfs_time)

datatable(tab, filter = "top")
```

**Show** 10 **entries** **Search:**

| pts_id | window_start_date | dfs_date | dfs_time |
|---|---|---|---|
| All | All | All | All |
| No data available in table | | | |

Showing 0 to 0 of 0 entries Previous Next

Hide

```
dt_survival <- dt_survival |>
  filter(dfs_time > 0)

aux <- dt |>
  select(pts_id, ct_dna_surveillance_available,
         window_start_date, dfs_date,
         surveillance_1_date:surveillance_12_date) |>
  mutate(across(.cols = surveillance_1_date:surveillance_12_date,
                .fns = ~ .x - window_start_date)) |>
  mutate(across(.cols = surveillance_1_date:surveillance_12_date,
                .fns = ~ .x < 0)) |>
  rowwise() |>
  mutate(sum_neg =
           sum(c_across(surveillance_1_date:surveillance_12_date),
               na.rm = TRUE))  |>
  select(pts_id, sum_neg)

tab <- left_join(aux, dt) |>
  filter(sum_neg > 0) |>
  select(pts_id, sum_neg, window_start_date,
         surveillance_1_date:surveillance_12_date) |>
  mutate(across(.cols = window_start_date:surveillance_12_date,
                .fns = ~ as_date(.x)))

datatable(tab, filter = "top")
```

Show 10 entries Search:

| pts_id | sum_neg | window_start_date | surveillance_1_date | surveillance_2_date | surveillance_3_date | surveillance_4_date |
|---|---|---|---|---|---|---|
| | All | All | All | All | All | All |

Showing 0 to 0 of 0 entries Previous Next

Hide

```
aux <- dt |>
  select(pts_id, ct_dna_surveillance_available,
         window_start_date, dfs_date,
         surveillance_1_date:surveillance_12_date) |>
  mutate(across(.cols = dfs_date:surveillance_12_date,
                .fns = ~ .x - window_start_date)) |>
  mutate(across(.cols = surveillance_2_date:surveillance_12_date,
                .fns = ~ dfs_date < .x)) |>
  rowwise() |>
  mutate(n_biomarker_after_event = sum(c_across(surveillance_2_date:
                                                  surveillance_12_date),
                                       na.rm = TRUE)) |>
  mutate(across(.cols = surveillance_1_date:surveillance_12_date,
                .fns = ~ !is.na(.x))) |>
  mutate(total_biomarker = sum(c_across(surveillance_2_date:
                                          surveillance_12_date),
                               na.rm = TRUE)) |>
  select(pts_id, n_biomarker_after_event, total_biomarker)

temp <- aux |>
  select(-pts_id) |>
  group_by(n_biomarker_after_event, total_biomarker) |>  # Direct grouping
  summarise(freq = n(), .groups = "drop")  # Drop groups after summarization


tab <- left_join(aux, dt) |>
  select(pts_id, n_biomarker_after_event, total_biomarker,
         dfs_date,
         surveillance_2_date:surveillance_12_date) |>
  mutate(across(.cols = dfs_date:surveillance_12_date,
                .fns = ~ as_date(.x))) |>
  filter(n_biomarker_after_event > 0)
datatable(tab, filter = "top")
```

Show 10 entries Search:

| | pts_id | n_biomarker_after_event | total_biomarker | dfs_date | surveillance_2_date | surveillance_3_date | surveillance_4_date |
|---|---|---|---|---|---|---|---|
| | | All | All | Al | All | All | All |
| 1 | UTUC-058 | 1 | 6 | 2025-03-24 | 2023-07-21 | 2023-10-13 | 2024-06-17 |
| 2 | UTUC-063 | 1 | 5 | 2025-02-25 | 2024-03-04 | 2024-06-07 | 2024-09-04 |
| 3 | UTUC-072 | 1 | 6 | 2025-03-24 | 2023-08-25 | 2023-12-07 | 2024-03-01 |
| 4 | UTUC-074 | 1 | 1 | 2025-03-19 | 2025-06-13 | | |

Showing 1 to 4 of 4 entries Previous 1 Next

Hide

```
aux <- tmerge(data1 = dt_survival,
              data2 = dt_survival,
              id = pts_id,
              dfs_event = event(dfs_time, dfs_event))
dt_final <- tmerge(data1 = aux,
                   data2 = dt_biomarker,
                   id = pts_id,
                   biomarker_status =
                     tdc(biomarker_time, biomarker_status))

datatable(dt_final, filter = "top")
```

Show 10 entries Search:

| | pts_id | dfs_time | dfs_event | tstart | tstop | biomarker_status |
|---|---|---|---|---|---|---|
| | All | All | All | All | All | All |
| 1 | UTUC-002 | 143 | 0 | 0 | 133 | |
| 2 | UTUC-002 | 143 | 1 | 133 | 143 | POSITIVE |
| 3 | UTUC-010 | 707 | 0 | 0 | 144 | |
| 4 | UTUC-010 | 707 | 0 | 144 | 181 | NEGATIVE |
| 5 | UTUC-010 | 707 | 0 | 181 | 223 | NEGATIVE |
| 6 | UTUC-010 | 707 | 0 | 223 | 267 | NEGATIVE |
| 7 | UTUC-010 | 707 | 0 | 267 | 307 | NEGATIVE |
| 8 | UTUC-010 | 707 | 0 | 307 | 349 | NEGATIVE |
| 9 | UTUC-010 | 707 | 0 | 349 | 392 | NEGATIVE |
| 10 | UTUC-010 | 707 | 0 | 392 | 433 | POSITIVE |

Showing 1 to 10 of 163 entries Previous 1 2 3 4 5 … 17 Next

Hide

```
# Syntax if there is not time-dependent covariate
# fit <- coxph(Surv(dfs_time, dfs_event) ~ biomarker_status,
#              data = dt_final)
# summary(fit)

fit <- coxph(Surv(tstart, tstop, dfs_event) ~ biomarker_status,
            data = dt_final)
summary(fit)
```

```
Call:
coxph(formula = Surv(tstart, tstop, dfs_event) ~ biomarker_status,
    data = dt_final)

  n= 127, number of events= 17
   (36 observations deleted due to missingness)

                            coef exp(coef) se(coef)     z Pr(>|z|)
biomarker_statusPOSITIVE  2.8209   16.7919   0.6524 4.324 1.53e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

                         exp(coef) exp(-coef) lower .95 upper .95
biomarker_statusPOSITIVE     16.79    0.05955     4.675     60.32

Concordance= 0.824  (se = 0.055 )
Likelihood ratio test= 26.23  on 1 df,   p=3e-07
Wald test            = 18.7  on 1 df,   p=2e-05
Score (logrank) test = 31  on 1 df,   p=3e-08
```

Hide

```
cox_fit_summary <- summary(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 = 16.79 (4.67-60.32); p = 0"
```

#Median numbers of time points in the longitudinal setting

Hide

```
# Load the dataset
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Clinical Data 102025.csv")
circ_data <- circ_data[circ_data$Final.cohort=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Surveillance!="",]
circ_datadf <- as.data.frame(circ_data)

median_Nsurvtps <- median(circ_datadf$Nsurvtps, na.rm = TRUE)
min_Nsurvtps <- min(circ_datadf$Nsurvtps, na.rm = TRUE)
max_Nsurvtps <- max(circ_datadf$Nsurvtps, na.rm = TRUE)

cat(sprintf("Median # of surveillance time points: %d (%d-%d)\n",
            median_Nsurvtps, min_Nsurvtps, max_Nsurvtps))
```

```
Median # of surveillance time points: 3 (1-12)
```

#Median of median interval of ctDNA timepoints and radiological imaging assessment

Hide

```
rm(list=ls())
setwd("~/Downloads")
df <- read.csv("RWE UTUC_OP 102025.csv", stringsAsFactors = FALSE)

names(df) <- trimws(names(df))

# ---- Helper: compute median interval per patient ----
median_interval_per_patient <- function(data, filter_col, filter_val) {
  data %>%
    filter(!!sym(filter_col) == filter_val) %>%
    arrange(PatientName, date.diff) %>%
    group_by(PatientName) %>%
    mutate(interval = date.diff - lag(date.diff)) %>%
    summarise(median_interval = median(interval, na.rm = TRUE), .groups = "drop") %>%
    mutate(event_group = filter_val)
}

# ---- Compute per-patient medians ----
ctDNA_patient_medians   <- median_interval_per_patient(df, "Event", "ctDNA")
imaging_patient_medians <- median_interval_per_patient(df, "Event_type", "Imaging")

# ---- Function to summarize patient-level medians to cohort-level ----
cohort_summary <- function(patient_data, event_label) {
  # Filter out patients with NA median intervals
  valid_data <- patient_data %>% filter(!is.na(median_interval))

  data.frame(
    Event_Type = event_label,
    Cohort_Median_Frequency = median(valid_data$median_interval, na.rm = TRUE),
    Min_Patient_Median = min(valid_data$median_interval, na.rm = TRUE),
    Max_Patient_Median = max(valid_data$median_interval, na.rm = TRUE),
    Range_Patient_Median = max(valid_data$median_interval, na.rm = TRUE) -
      min(valid_data$median_interval, na.rm = TRUE)
  )
}

# ---- Combine all results ----
results <- bind_rows(
  cohort_summary(ctDNA_patient_medians, "ctDNA"),
  cohort_summary(imaging_patient_medians, "Imaging")
)

# ---- Print cohort-level summary ----
cat("===== Median Frequency (Days) per Cohort =====\n")
```

```
===== Median Frequency (Days) per Cohort =====
```

Hide

```
print(results, row.names = FALSE)
```

```
 Event_Type Cohort_Median_Frequency Min_Patient_Median Max_Patient_Median Range_Patient_Median
      ctDNA                   75.75                 14                156                  142
    Imaging                  154.25                  0                932                  932
```

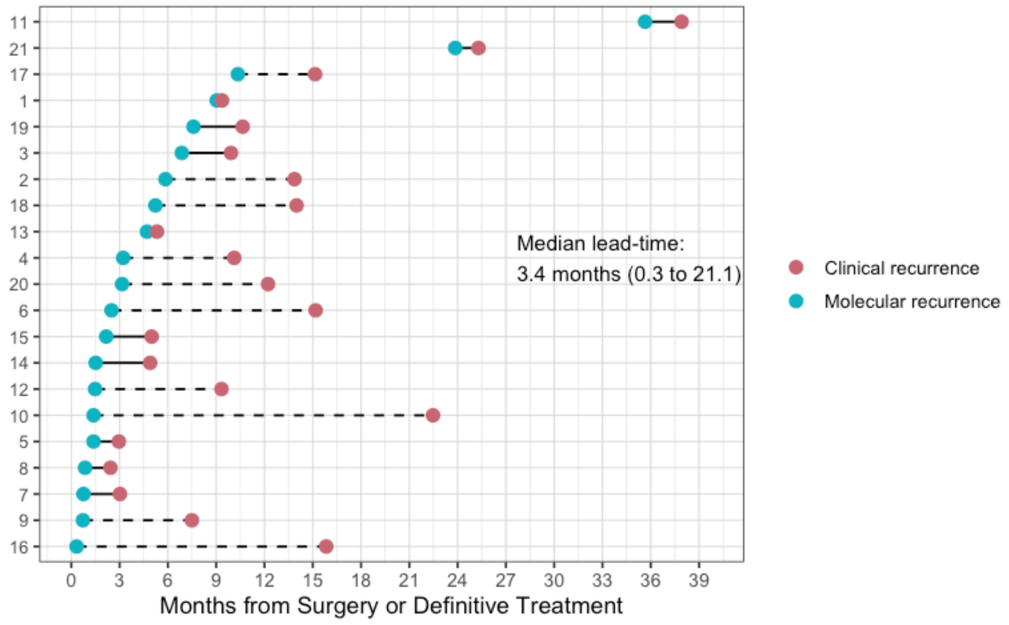
Hide

```
# ---- OPTIONAL: Save patient-level medians ----
patient_level_medians <- bind_rows(ctDNA_patient_medians, imaging_patient_medians)
#write.csv(patient_level_medians, "patient_median_intervals.csv", row.names = FALSE)
```

#Plot for individual lead-time calculations for each pt

Hide

```
rm(list=ls())
csv_path <- "~/Downloads/CLIA UTUC_Lead Time pts.csv"
df <- read.csv(csv_path, check.names = FALSE)
if ("Final.cohort" %in% names(df)) {
  df <- df[df$Final.cohort == TRUE, ]
} else {
  warning("Column 'Final.cohort' not found in the dataset.")
}
id_candidates <- c("Patient","ID","Pt","Subject","Sample")
id_col <- id_candidates[id_candidates %in% names(df)][1]
if (is.na(id_col)) {
  df <- df %>% mutate(Patient = row_number())
  id_col <- "Patient"
}

num_cols <- intersect(c("MR","CR","LT"), names(df))
df[num_cols] <- lapply(df[num_cols], function(x) suppressWarnings(as.numeric(x)))

# If LT missing, compute in days
if (!("LT" %in% names(df))) {
  df <- df %>% mutate(LT = CR - MR)
}

# ---- convert to months ----
# Approximate: 30.44 days per month (average)
days_to_months <- function(x) x / 30.437

df <- df %>%
  mutate(MR_mo = days_to_months(MR),
         CR_mo = days_to_months(CR),
         LT_mo = days_to_months(LT))

# ---- ordering for y-axis ----
df <- df %>%
  mutate(Earliest = pmin(MR_mo, CR_mo, na.rm = TRUE)) %>%
  arrange(Earliest) %>%
  mutate(Patient_f = factor(.data[[id_col]], levels = .data[[id_col]]))

# ---- long format for points ----
points_long <- df %>%
  select(Patient_f, MR_mo, CR_mo) %>%
  pivot_longer(c(MR_mo, CR_mo), names_to = "Type", values_to = "Months") %>%
  mutate(Type = dplyr::recode(Type,
                              "MR_mo" = "Molecular recurrence",
                              "CR_mo" = "Clinical recurrence"))

# ---- segments between MR and CR ----
segments_df <- df %>%
  transmute(Patient_f,
            x0 = MR_mo, x1 = CR_mo,
            lt_flag = if_else(LT > 120, "gt120", "le120"))

# ---- annotation ----
med_lt  <- median(df$LT_mo, na.rm = TRUE)
min_lt  <- min(df$LT_mo, na.rm = TRUE)
max_lt  <- max(df$LT_mo, na.rm = TRUE)

annot_label <- sprintf("Median lead-time:\n%.1f months (%.1f to %.1f)",
                       med_lt, min_lt, max_lt)

# ---- plot ----
pal <- c("Molecular recurrence" = "#10B4C1",
         "Clinical recurrence"  = "#C96A72")

x_max <- max(c(df$MR_mo, df$CR_mo), na.rm = TRUE)
y_mid <- levels(df$Patient_f)[ceiling(nlevels(df$Patient_f) * 0.55)]

p <- ggplot() +
  geom_segment(data = segments_df,
               aes(x = x0, xend = x1, y = Patient_f, yend = Patient_f,
                   linetype = lt_flag),
               linewidth = 0.6, color = "black") +
  scale_linetype_manual(values = c(le120 = "solid", gt120 = "dashed"),
                        guide = "none") +
  geom_point(data = points_long,
             aes(x = Months, y = Patient_f, color = Type),
             size = 2.8) +
  scale_color_manual(values = pal, name = NULL) +
  labs(x = "Months from Surgery or Definitive Treatment", y = NULL) +
  annotate("text",
           x = x_max * 0.73,
           y = y_mid,
           label = annot_label,
           hjust = 0, vjust = 0.5, size = 3.8) +
  scale_x_continuous(breaks = seq(0, ceiling(x_max/3)*3, by = 3)) +
  coord_cartesian(xlim = c(0, x_max * 1.05)) +
  theme_bw(base_size = 12) +
  theme(
    panel.grid.major = element_line(color = "grey85", linewidth = 0.3),
    panel.grid.minor = element_line(color = "grey92", linewidth = 0.2),
    axis.text.y = element_text(size = 9)
  )

print(p)
```



Hide

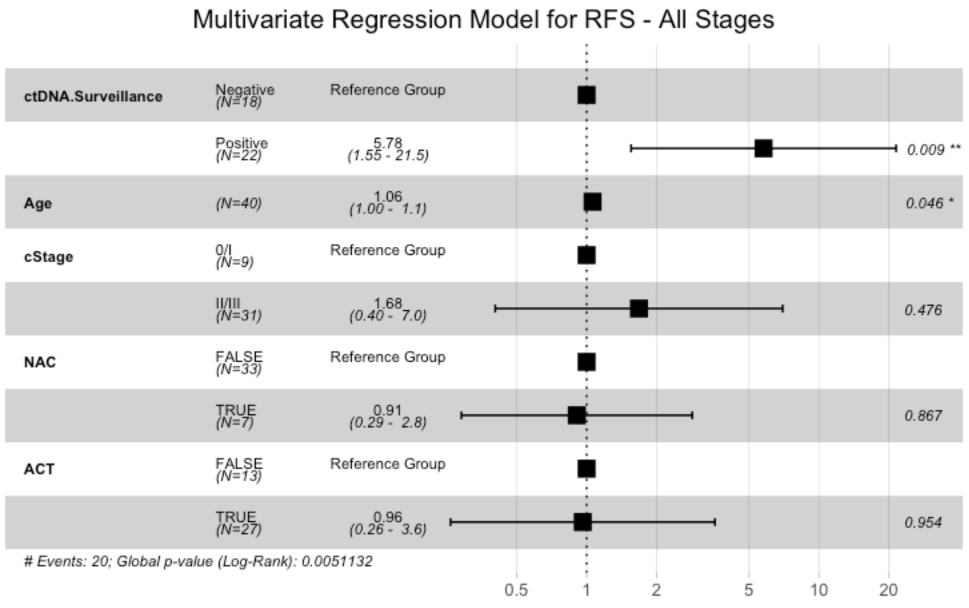
```
wc_df <- df %>% dplyr::select(MR, CR) %>% tidyr::drop_na()
paired_diff <- wc_df$CR - wc_df$MR

wilx <- wilcox.test(
  x = wc_df$CR, y = wc_df$MR,
  paired = TRUE,
  alternative = "two.sided",
  conf.int = TRUE,      # gives CI for the Hodges–Lehmann estimate of the median difference
  exact = FALSE         # safer for ties/large N
)

W_stat   <- unname(wilx$statistic)          # Wilcoxon V
p_value  <- wilx$p.value
HL_est   <- unname(wilx$estimate)           # median of (CR − MR)
HL_ci    <- wilx$conf.int                   # CI for median difference

# Simple p-value formatter for annotation/publication
fmt_p <- function(p) {
  if (is.na(p)) return("NA")
  if (p < 0.001) "< 0.001" else sprintf("= %.3f", round(p, 3))
}
p_text <- paste0("P ", fmt_p(p_value))  # e.g., "P = 0.003" or "P < 0.001"

# Optional: print a compact summary
cat("\nPaired Wilcoxon signed-rank test (CR vs MR)\n",
    "--------------------------------------------\n",
    sprintf("N pairs: %d", nrow(wc_df)), "\n",
    sprintf("W (V): %g", W_stat), "\n",
    sprintf("P-value: %s", ifelse(p_value < 0.001, "< 0.001", sprintf("%.6f", p_value))), "\n",
    sprintf("Hodges–Lehmann median difference (CR − MR): %.1f days", HL_est), "\n",
    sprintf("95%% CI: [%.1f, %.1f] days", HL_ci[1], HL_ci[2]), "\n", sep = "")
```

```
Paired Wilcoxon signed–rank test (CR vs MR)
–––––––––––––––––––––––––––––––––––––––––––
N pairs: 21
W (V): 231
P–value: < 0.001
Hodges–Lehmann median difference (CR – MR): 155.5 days
95% CI: [86.0, 241.5] days
```

#Multivariate cox regression at Surveillance Window for RFS

Hide

```
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Clinical Data 102025.csv")
circ_data <- circ_data[circ_data$Final.cohort=="TRUE",]
circ_data <- circ_data[circ_data$ctDNA.Surveillance!="",]

circ_data$ctDNA.Surveillance <- factor(circ_data$ctDNA.Surveillance, levels=c("NEGATIVE","POSITIVE"), labels = c
("Negative", "Positive"))
circ_data$cStage <- factor(circ_data$cStage, levels = c("0/I", "II/III"))
circ_data$NAC <- factor(circ_data$NAC, levels = c("FALSE", "TRUE"))
circ_data$ACT <- factor(circ_data$ACT, levels = c("FALSE", "TRUE"))
surv_object <- Surv(time = circ_data$RFS.months, event = circ_data$RFS.Event)
cox_fit <- coxph(surv_object ~ ctDNA.Surveillance + Age + cStage + NAC + ACT, data=circ_data)
ggforest(cox_fit, data = circ_data, main = "Multivariate Regression Model for RFS – All Stages", refLabel = "Refe
rence Group")
```



Hide

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

#ctDNA and MTM/mL Dynamics for pts at surveillance window

Hide

```
#Dynamics and MTM/mL plots for patients with ctDNA negative at surveillance
rm(list=ls())
setwd("~/Downloads")
df <- read.csv("CLIA UTUC ctDNA MTM.csv", stringsAsFactors = FALSE)
df <- df[df$Final.cohort=="TRUE",]
df <- df[df$ctDNA.Surveillance=="NEGATIVE",]

df$RFS.Event <- ifelse(df$RFS.Event %in% c("No", "no", "FALSE", "False", "0"), FALSE,
                       ifelse(df$RFS.Event %in% c("Yes", "yes", "TRUE", "True", "1"), TRUE, NA))
df$RFS.Event <- factor(df$RFS.Event, levels = c(FALSE, TRUE))
df <- df %>%
  group_by(PatientName) %>%
  filter(n() >= 2) %>% #keep only pts with at least 2 post-surgery time points
  ungroup()

num_unique <- length(unique(df$PatientName))
cat("Number of unique patients:", num_unique, "\n")
```

```
Number of unique patients: 17
```

Hide

```
df_patient_pfs <- df %>%
  group_by(PatientName) %>%
  dplyr::summarize(
    PFS_True = any(RFS.Event == TRUE, na.rm = TRUE),
    PFS_False = all(RFS.Event == FALSE, na.rm = TRUE)
  )

num_true <- sum(df_patient_pfs$PFS_True)
num_false <- sum(df_patient_pfs$PFS_False)

cat("Number of unique patients with Event:", num_true, "\n")
```

```
Number of unique patients with Event: 3
```

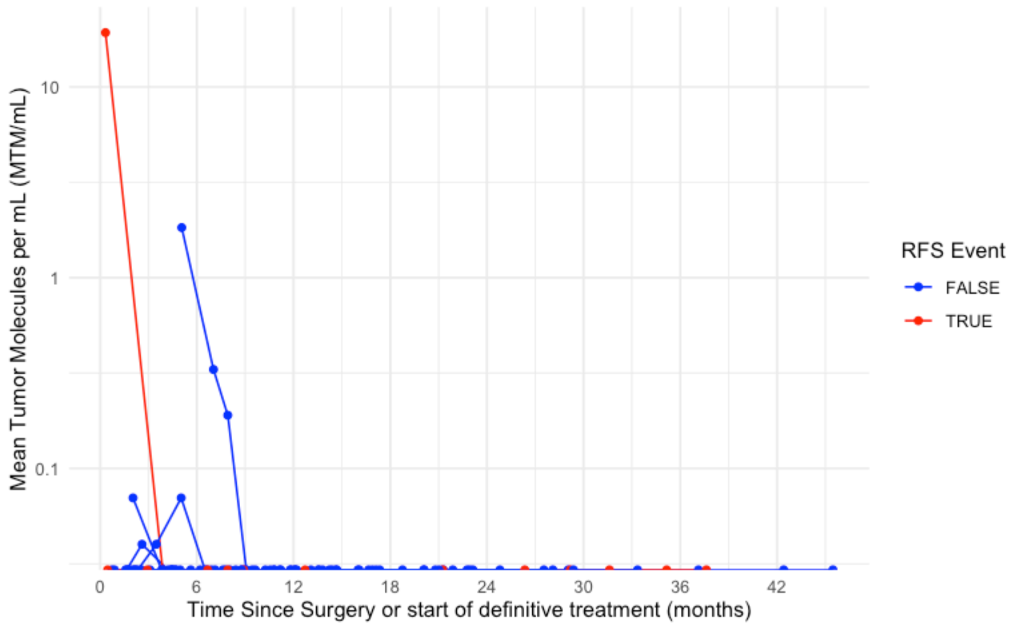
Hide

```
cat("Number of unique patients with No Event:", num_false, "\n")
```

```
Number of unique patients with No Event: 14
```

Hide

```
p <- ggplot(df, aes(x = date.diff.months,
                    y = MTM.mL,
                    group = PatientName,
                    color = RFS.Event)) +
  geom_line() +      # Connect timepoints for each patient
  geom_point() +     # Add points for each timepoint
  # Use a log10 scale for the y-axis with specified breaks
  scale_y_log10(breaks = c(0.01, 0.1, 1, 10, 100, 1000),
                labels = c("0.01","0.1", "1", "10", "100", "1000")) +
  scale_x_continuous(breaks = seq(0, max(df$date.diff.months, na.rm = TRUE), by = 6)) +
  scale_color_manual(values = c("FALSE" = "blue", "TRUE" = "red")) +
  labs(
    x = "Time Since Surgery or start of definitive treatment (months)",
    y = "Mean Tumor Molecules per mL (MTM/mL)",
    color = "RFS Event"
  ) +
  theme_minimal()
print(p)
```



Hide

```

#Dynamics and MTM/mL plots for patients with ctDNA positive at surveillance
rm(list=ls())
setwd("~/Downloads")
df <- read.csv("CLIA UTUC ctDNA MTM.csv", stringsAsFactors = FALSE)
df <- df[df$Final.cohort=="TRUE",]
df <- df[df$ctDNA.Surveillance=="POSITIVE",]

df$RFS.Event <- ifelse(df$RFS.Event %in% c("No", "no", "FALSE", "False", "0"), FALSE,
                       ifelse(df$RFS.Event %in% c("Yes", "yes", "TRUE", "True", "1"), TRUE, NA))
df$RFS.Event <- factor(df$RFS.Event, levels = c(FALSE, TRUE))
df <- df %>%
  group_by(PatientName) %>%
  filter(n() >= 2) %>% #keep only pts with at least 2 post-surgery time points
  ungroup()

num_unique <- length(unique(df$PatientName))
cat("Number of unique patients:", num_unique, "\n")
```

```
Number of unique patients: 21
```

Hide

```r
df_patient_pfs <- df %>%
  group_by(PatientName) %>%
  dplyr::summarize(
    PFS_True = any(RFS.Event == TRUE, na.rm = TRUE),
    PFS_False = all(RFS.Event == FALSE, na.rm = TRUE)
  )

num_true <- sum(df_patient_pfs$PFS_True)
num_false <- sum(df_patient_pfs$PFS_False)

cat("Number of unique patients with Event:", num_true, "\n")
```

```
Number of unique patients with Event: 16
```

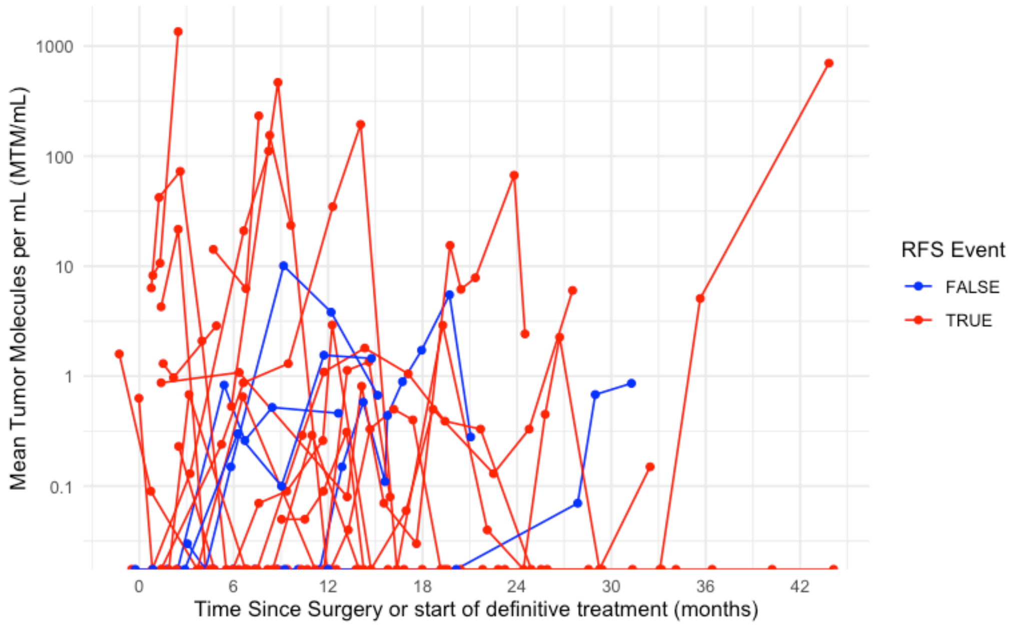
Hide

```r
cat("Number of unique patients with No Event:", num_false, "\n")
```

```
Number of unique patients with No Event: 5
```

Hide

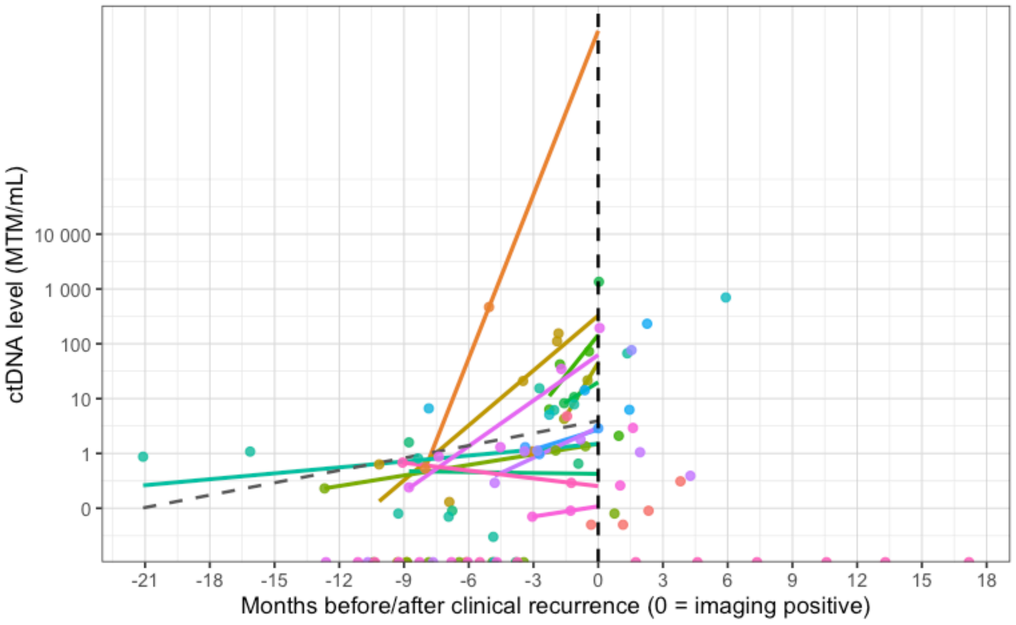
```r
p <- ggplot(df, aes(x = date.diff.months,
                    y = MTM.mL,
                    group = PatientName,
                    color = RFS.Event)) +
  geom_line() +      # Connect timepoints for each patient
  geom_point() +     # Add points for each timepoint
  # Use a log10 scale for the y-axis with specified breaks
  scale_y_log10(breaks = c(0.01, 0.1, 1, 10, 100, 1000),
                labels = c("0.01","0.1", "1", "10", "100", "1000")) +
  scale_x_continuous(breaks = seq(0, max(df$date.diff.months, na.rm = TRUE), by = 6)) +
  scale_color_manual(values = c("FALSE" = "blue", "TRUE" = "red")) +
  labs(
    x = "Time Since Surgery or start of definitive treatment (months)",
    y = "Mean Tumor Molecules per mL (MTM/mL)",
    color = "RFS Event"
  ) +
  theme_minimal()
print(p)
```



#ctDNA velocity and lead time liner regression

Hide

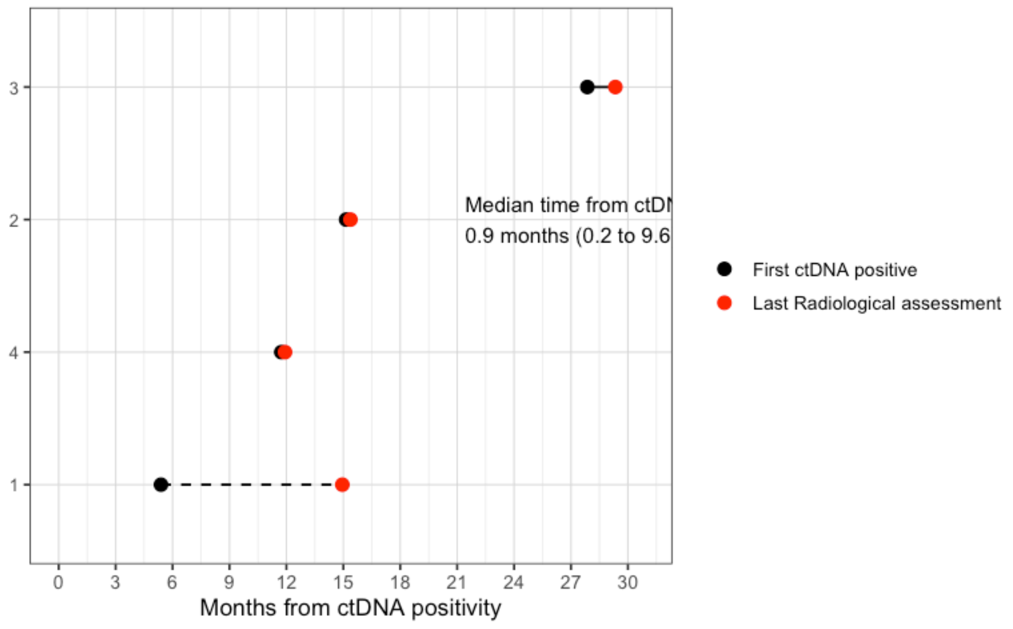
```r
rm(list=ls())
csv_path <- "~/Downloads/CLIA UTUC_ctDNA velocity.csv"  # <- adjust if needed
df_raw <- read.csv(csv_path, check.names = FALSE)
df <- df_raw %>%
  rename(MTM_mL = `MTM.mL`) %>%
  mutate(
    daysCR.months = as.numeric(daysCR.months),
    MTM_mL = as.numeric(MTM_mL)
  ) %>%
  filter(Final.cohort == TRUE, !is.na(PatientName), !is.na(daysCR.months), !is.na(MTM_mL))

preCR <- df %>%
  filter(daysCR.months <= 0, is.finite(MTM_mL), MTM_mL > 0)

eligible <- preCR %>%
  group_by(PatientName) %>%
  filter(n() >= 2, n_distinct(daysCR.months) >= 2) %>%
  ungroup()
if (nrow(eligible) == 0) {
  warning("No patients have ≥2 valid pre-recurrence points with distinct times; regression lines will be omitte
d.")
}

fits <- eligible %>%
  group_by(PatientName) %>%
  summarise(x_min = min(daysCR.months, na.rm = TRUE), .groups = "drop") %>%
  mutate(grid = map(x_min, ~seq(.x, 0, length.out = 50))) %>%
  select(PatientName, grid)

predict_patient <- function(dat, newx) {
  if (length(newx) == 0) {
    return(tibble(daysCR.months = numeric(0), MTM_mL = numeric(0)))
  }
  dat2 <- dat %>%
    filter(is.finite(MTM_mL), MTM_mL > 0) %>%
    mutate(log_ctdna = log10(MTM_mL))
  if (nrow(dat2) < 2 || n_distinct(dat2$daysCR.months) < 2 || any(!is.finite(dat2$log_ctdna))) {
    return(tibble(daysCR.months = numeric(0), MTM_mL = numeric(0)))
  }
  m <- lm(log_ctdna ~ daysCR.months, data = dat2)
  tibble(
    daysCR.months = newx,
    MTM_mL = 10 ^ predict(m, newdata = tibble(daysCR.months = newx))
  )
}

pred_lines <- eligible %>%
  group_by(PatientName) %>%
  tidyr::nest() %>%                        # list-column "data" per patient
  left_join(fits, by = "PatientName") %>% # list-column "grid" per patient
  mutate(pred = map2(data, grid, ~predict_patient(.x, .y))) %>%
  select(PatientName, pred) %>%
  tidyr::unnest(pred)

pooled_line <- {
  if (nrow(preCR) >= 2 && n_distinct(preCR$daysCR.months) >= 2) {
    pooled_x <- seq(min(preCR$daysCR.months, na.rm = TRUE), 0, length.out = 100)
    pooled_fit <- lm(log10(MTM_mL) ~ daysCR.months, data = preCR)
    tibble(
      daysCR.months = pooled_x,
      MTM_mL = 10 ^ predict(pooled_fit, newdata = tibble(daysCR.months = pooled_x))
    )
  } else {
    tibble(daysCR.months = numeric(0), MTM_mL = numeric(0))
  }
}

x_min <- floor(min(df$daysCR.months, na.rm = TRUE) / 3) * 3
x_max <- ceiling(max(df$daysCR.months, na.rm = TRUE) / 3) * 3
y_min_pos <- max(min(df$MTM_mL[df$MTM_mL > 0], na.rm = TRUE) / 2, 0.01)
y_max_pos <- 10 ^ ceiling(log10(max(df$MTM_mL, na.rm = TRUE)))
log_breaks <- 10 ^ seq(floor(log10(y_min_pos)), ceiling(log10(y_max_pos)))

p <- ggplot() +
  # per-patient fitted lines (if any)
  geom_line(data = pred_lines,
            aes(x = daysCR.months, y = MTM_mL, color = PatientName),
            linewidth = 1) +
  # pooled dashed trend (if any)
  geom_line(data = pooled_line,
            aes(x = daysCR.months, y = MTM_mL),
            linewidth = 0.8, linetype = "dashed", color = "grey35") +
  # raw points (all timepoints, before and after)
  geom_point(data = df,
             aes(x = daysCR.months, y = MTM_mL, color = PatientName),
             size = 1.8, alpha = 0.85) +
  # vertical line at imaging-positive date
  geom_vline(xintercept = 0, linetype = "dashed", linewidth = 0.8, color = "black") +
  scale_y_log10(breaks = log_breaks,
                labels = label_number(accuracy = 1)) +
  scale_x_continuous(breaks = seq(x_min, x_max, by = 3)) +  # every 3 months
  labs(
    x = "Months before/after clinical recurrence (0 = imaging positive)",
    y = "ctDNA level (MTM/mL)",
    color = "Patient"
  ) +
  theme_bw(base_size = 12) +
  theme(
    panel.grid.major = element_line(color = "grey85", linewidth = 0.3),
    panel.grid.minor = element_line(color = "grey92", linewidth = 0.2),
    legend.position = "none"  # change to "right" if you want the legend
  )

print(p)
```



#Time from first ctDNA positive timepoint to last imaging in surveillance_False Positive patients

Hide

```r
rm(list=ls())
csv_path <- "~/Downloads/CLIA UTUC_Time from ctDNA to imaging FP pts.csv"
df <- read.csv(csv_path, check.names = FALSE)
if ("Final.cohort" %in% names(df)) {
  df <- df[df$Final.cohort == TRUE, ]
} else {
  warning("Column 'Final.cohort' not found in the dataset.")
}
id_candidates <- c("Patient","ID","Pt","Subject","Sample")
id_col <- id_candidates[id_candidates %in% names(df)][1]
if (is.na(id_col)) {
  df <- df %>% mutate(Patient = row_number())
  id_col <- "Patient"
}

num_cols <- intersect(c("MR","CR","LT"), names(df))
df[num_cols] <- lapply(df[num_cols], function(x) suppressWarnings(as.numeric(x)))

# If LT missing, compute in days
if (!("LT" %in% names(df))) {
  df <- df %>% mutate(LT = CR - MR)
}

# ---- convert to months ----
# Approximate: 30.44 days per month (average)
days_to_months <- function(x) x / 30.437

df <- df %>%
  mutate(MR_mo = days_to_months(MR),
         CR_mo = days_to_months(CR),
         LT_mo = days_to_months(LT))

# ---- ordering for y-axis ----
df <- df %>%
  mutate(Earliest = pmin(MR_mo, CR_mo, na.rm = TRUE)) %>%
  arrange(Earliest) %>%
  mutate(Patient_f = factor(.data[[id_col]], levels = .data[[id_col]]))

# ---- long format for points ----
points_long <- df %>%
  select(Patient_f, MR_mo, CR_mo) %>%
  pivot_longer(c(MR_mo, CR_mo), names_to = "Type", values_to = "Months") %>%
  mutate(Type = dplyr::recode(Type,
                              "MR_mo" = "First ctDNA positive",
                              "CR_mo" = "Last Radiological assessment"))

# ---- segments between MR and CR ----
segments_df <- df %>%
  transmute(Patient_f,
            x0 = MR_mo, x1 = CR_mo,
            lt_flag = if_else(LT > 120, "gt120", "le120"))

# ---- annotation ----
med_lt  <- median(df$LT_mo, na.rm = TRUE)
min_lt  <- min(df$LT_mo, na.rm = TRUE)
max_lt  <- max(df$LT_mo, na.rm = TRUE)

annot_label <- sprintf("Median time from ctDNA positive to last imaging:\n%.1f months (%.1f to %.1f)",
                       med_lt, min_lt, max_lt)

# ---- plot ----
pal <- c("First ctDNA positive" = "black",
         "Last Radiological assessment"  = "red")

x_max <- max(c(df$MR_mo, df$CR_mo), na.rm = TRUE)
y_mid <- levels(df$Patient_f)[ceiling(nlevels(df$Patient_f) * 0.55)]

p <- ggplot() +
  geom_segment(data = segments_df,
               aes(x = x0, xend = x1, y = Patient_f, yend = Patient_f,
                   linetype = lt_flag),
               linewidth = 0.6, color = "black") +
  scale_linetype_manual(values = c(le120 = "solid", gt120 = "dashed"),
                        guide = "none") +
  geom_point(data = points_long,
             aes(x = Months, y = Patient_f, color = Type),
             size = 2.8) +
  scale_color_manual(values = pal, name = NULL) +
  labs(x = "Months from ctDNA positivity", y = NULL) +
  annotate("text",
           x = x_max * 0.73,
           y = y_mid,
           label = annot_label,
           hjust = 0, vjust = 0.5, size = 3.8) +
  scale_x_continuous(breaks = seq(0, ceiling(x_max/3)*3, by = 3)) +
  coord_cartesian(xlim = c(0, x_max * 1.05)) +
  theme_bw(base_size = 12) +
  theme(
    panel.grid.major = element_line(color = "grey85", linewidth = 0.3),
    panel.grid.minor = element_line(color = "grey92", linewidth = 0.2),
    axis.text.y = element_text(size = 9)
  )

print(p)
```



Hide

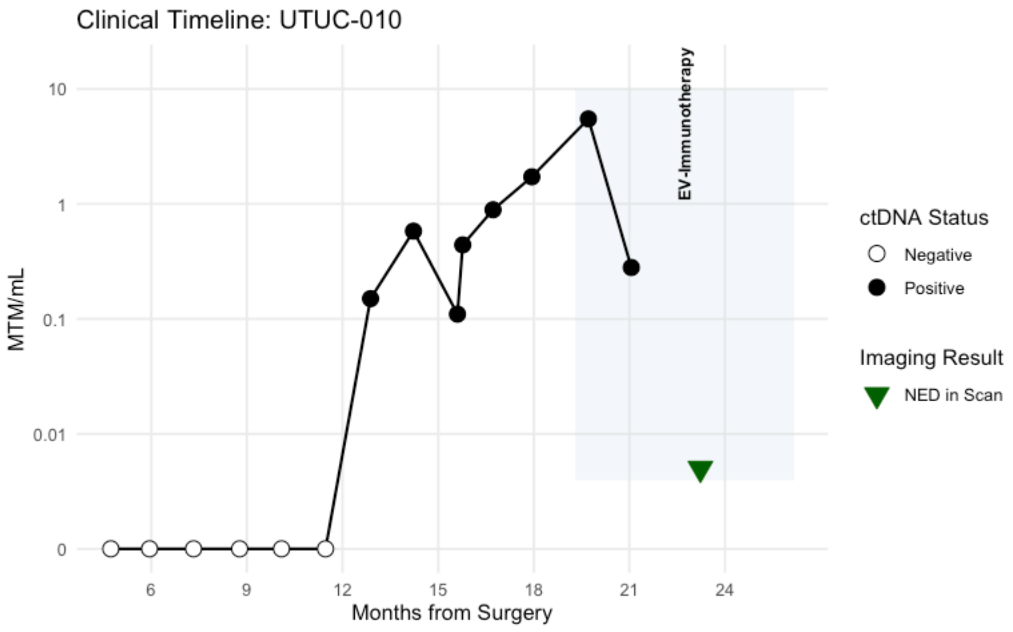
```
wc_df <- df %>% dplyr::select(MR, CR) %>% tidyr::drop_na()
paired_diff <- wc_df$CR - wc_df$MR

wilx <- wilcox.test(
  x = wc_df$CR, y = wc_df$MR,
  paired = TRUE,
  alternative = "two.sided",
  conf.int = TRUE,      # gives CI for the Hodges–Lehmann estimate of the median difference
  exact = FALSE         # safer for ties/large N
)

W_stat   <- unname(wilx$statistic)          # Wilcoxon V
p_value  <- wilx$p.value
HL_est   <- unname(wilx$estimate)           # median of (CR - MR)
HL_ci    <- wilx$conf.int                   # CI for median difference

# Simple p-value formatter for annotation/publication
fmt_p <- function(p) {
  if (is.na(p)) return("NA")
  if (p < 0.001) "< 0.001" else sprintf("= %.3f", round(p, 3))
}
p_text <- paste0("P ", fmt_p(p_value))  # e.g., "P = 0.003" or "P < 0.001"

# Optional: print a compact summary
cat("\nPaired Wilcoxon signed-rank test (CR vs MR)\n",
    "--------------------------------------------\n",
    sprintf("N pairs: %d", nrow(wc_df)), "\n",
    sprintf("W (V): %g", W_stat), "\n",
    sprintf("P-value: %s", ifelse(p_value < 0.001, "< 0.001", sprintf("%.6f", p_value))), "\n",
    sprintf("Hodges–Lehmann median difference (CR - MR): %.1f days", HL_est), "\n",
    sprintf("95%% CI: [%.1f, %.1f] days", HL_ci[1], HL_ci[2]), "\n", sep = "")
```

```
Paired Wilcoxon signed-rank test (CR vs MR)
-------------------------------------------
N pairs: 4
W (V): 10
P-value: 0.100348
Hodges–Lehmann median difference (CR – MR): 27.7 days
95% CI: [6.0, 291.0] days
```

#Individual Pt Plots_False Positive patients

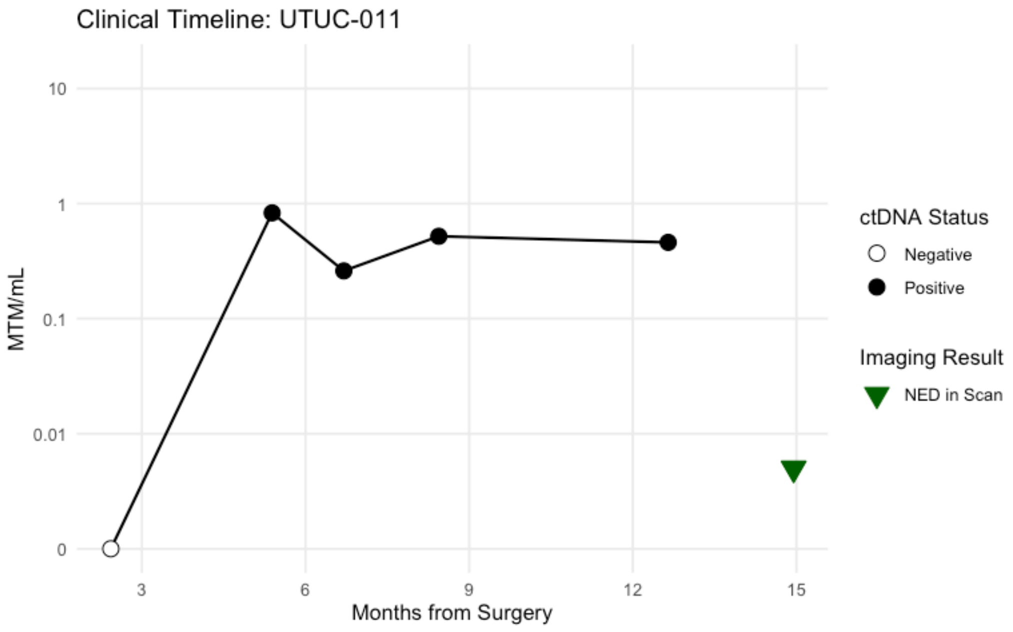
Hide

```r
#Filter for Patient UTUC-010
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Pt Specific Plot data.csv")
plot_data <- circ_data %>%
  filter(PatientName == "UTUC-010")

# 2. Process layers
# Handle ctDNA log-scale pseudo-zero
data_Signatera <- plot_data %>%
  filter(Event_type %in% c("ctDNA_pos", "ctDNA_neg")) %>%
  mutate(MTM_Log = ifelse(MTM.mL == 0, 0.001, MTM.mL))

data_Imaging <- plot_data %>%
  filter(Event_type == "Imaging")

# Treatment blocks
data_Treatment <- plot_data %>%
  filter(!is.na(Tx_type) & Tx_type != "NA" & !is.na(Tx_start.months)) %>%
  distinct(Tx_type, Tx_start.months, Tx_end.months)

# 3. Create the Plot
ggplot() +
  # Treatment Rectangles
  geom_rect(data = data_Treatment,
            aes(xmin = Tx_start.months, xmax = Tx_end.months, ymin = 0.004, ymax = 10),
            fill = "lightblue", alpha = 0.15) +
  geom_text(data = data_Treatment,
            aes(x = (Tx_start.months + Tx_end.months)/2, y = 5, label = Tx_type),
            angle = 90, color = "black", size = 3, fontface = "bold") +

  # ctDNA Line
  geom_line(data = data_Signatera,
            aes(x = date.diff.months, y = MTM_Log), color = "black", linewidth = 0.7) +

  # ctDNA Points (Mapped to Fill)
  geom_point(data = data_Signatera,
             aes(x = date.diff.months, y = MTM_Log, fill = Event_type),
             shape = 21, size = 3.5, color = "black") +

  # Imaging Points (Mapped to Shape and Color to create a second legend)
  geom_point(data = data_Imaging,
             aes(x = date.diff.months, y = 0.005, color = "NED in Scan"),
             shape = 25, size = 4, fill = "darkgreen") +

  # X-Axis: 3-month intervals
  scale_x_continuous(breaks = seq(0, max(plot_data$date.diff.months, na.rm=T) + 3, by = 3)) +

  # Y-Axis: Log10 scale
  scale_y_log10(breaks = c(0.001, 0.01, 0.1, 1, 10),
                labels = c("0", "0.01", "0.1", "1", "10"),
                limits = c(0.001, 15)) +

  # Legend for ctDNA
  scale_fill_manual(values = c("ctDNA_pos" = "black", "ctDNA_neg" = "white"),
                    labels = c("Negative", "Positive"),
                    name = "ctDNA Status") +

  # Legend for Imaging
  scale_color_manual(values = c("NED in Scan" = "darkgreen"),
                     name = "Imaging Result") +

  # Styling the legend to show the triangle correctly
  guides(color = guide_legend(override.aes = list(shape = 25, fill = "darkgreen"))) +

  labs(x = "Months from Surgery",
       y = "MTM/mL",
       title = "Clinical Timeline: UTUC-010") +
  theme_minimal() +
  theme(panel.grid.minor = element_blank(),
        legend.position = "right")
```


Clinical Timeline: UTUC-010
MTM/mL
10
1
0.1
0.01
0
6
9
12
15
18
21
24
Months from Surgery
EV-Immunotherapy
ctDNA Status
Negative
Positive
Imaging Result
NED in Scan

Hide

```
#Filter for Patient UTUC-011
rm(list=ls())
setwd("~/Downloads")
circ_data <- read.csv("RWE UTUC_Pt Specific Plot data.csv")
plot_data <- circ_data %>%
  filter(PatientName == "UTUC-011")

# 2. Process layers
# Handle ctDNA log-scale pseudo-zero
data_Signatera <- plot_data %>%
  filter(Event_type %in% c("ctDNA_pos", "ctDNA_neg")) %>%
  mutate(MTM_Log = ifelse(MTM.mL == 0, 0.001, MTM.mL))

data_Imaging <- plot_data %>%
  filter(Event_type == "Imaging")

# Treatment blocks
data_Treatment <- plot_data %>%
  filter(!is.na(Tx_type) & Tx_type != "NA" & !is.na(Tx_start.months)) %>%
  distinct(Tx_type, Tx_start.months, Tx_end.months)

# 3. Create the Plot
ggplot() +
  # Treatment Rectangles
  geom_rect(data = data_Treatment,
            aes(xmin = Tx_start.months, xmax = Tx_end.months, ymin = 0.004, ymax = 10),
            fill = "lightblue", alpha = 0.15) +
  geom_text(data = data_Treatment,
            aes(x = (Tx_start.months + Tx_end.months)/2, y = 5, label = Tx_type),
            angle = 90, color = "black", size = 3, fontface = "bold") +

  # ctDNA Line
  geom_line(data = data_Signatera,
            aes(x = date.diff.months, y = MTM_Log), color = "black", linewidth = 0.7) +

  # ctDNA Points (Mapped to Fill)
  geom_point(data = data_Signatera,
             aes(x = date.diff.months, y = MTM_Log, fill = Event_type),
             shape = 21, size = 3.5, color = "black") +

  # Imaging Points (Mapped to Shape and Color to create a second legend)
  geom_point(data = data_Imaging,
             aes(x = date.diff.months, y = 0.005, color = "NED in Scan"),
             shape = 25, size = 4, fill = "darkgreen") +

  # X-Axis: 3-month intervals
  scale_x_continuous(breaks = seq(0, max(plot_data$date.diff.months, na.rm=T) + 3, by = 3)) +

  # Y-Axis: Log10 scale
  scale_y_log10(breaks = c(0.001, 0.01, 0.1, 1, 10),
                labels = c("0", "0.01", "0.1", "1", "10"),
                limits = c(0.001, 15)) +

  # Legend for ctDNA
  scale_fill_manual(values = c("ctDNA_pos" = "black", "ctDNA_neg" = "white"),
                    labels = c("Negative", "Positive"),
                    name = "ctDNA Status") +

  # Legend for Imaging
  scale_color_manual(values = c("NED in Scan" = "darkgreen"),
                     name = "Imaging Result") +

  # Styling the legend to show the triangle correctly
  guides(color = guide_legend(override.aes = list(shape = 25, fill = "darkgreen"))) +

  labs(x = "Months from Surgery",
       y = "MTM/mL",
       title = "Clinical Timeline: UTUC-011") +
  theme_minimal() +
  theme(panel.grid.minor = element_blank(),
        legend.position = "right")
```


Clinical Timeline: UTUC-011
10
1
0.1
0.01
0
MTM/mL
3
6
9
12
15
Months from Surgery
ctDNA Status
Negative
Positive
Imaging Result
NED in Scan