Survival Analysis with HGAT group and telomerase status

Run Jin

2/4/2022

This notebook will do the following survival analysis

1. Univariate analysis

- DMG H3K28 vs rest
- ALT vs. non-ALT for all samples
- Telhunt score (separate into categories by 1.07)
- CCA telhunt category

2. Multivariate analysis

- DMG H3K28 vs rest + ALT vs. non-ALT
- DMG H3K28 vs rest + ALT vs. non-ALT + sex + ATRX (mut N/Y)
- DMG H3K28 vs rest + Telhunt score (categorical) + sex + ATRX (mut N/Y)
- DMG H3K28 vs rest + Telhunt score (continuous) + sex + ATRX (mut N/Y)
- DMG H3K28 vs rest + CCA Telhunt category + sex + ATRX (mut N/Y)

Packages and functions Read in set up script.

```
library(survival)
library(dplyr)
```

```
##
## Attaching package: 'dplyr'

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
library(ggplot2)
library(ggpubr)
```

Set up directories

```
root_dir <- rprojroot::find_root(rprojroot::has_dir(".git"))
analysis_dir <- file.path(root_dir, "analyses", "survival-analysis")

plots_dir <- file.path(analysis_dir, "plots")
if (!dir.exists(plots_dir)) {
  dir.create(plots_dir)
}

output_dir <- file.path(analysis_dir, "output")
if (!dir.exists(output_dir)) {
  dir.create(plots_dir)
}
```

Read in files

```
# get the meta information
meta <- readr::read_tsv(file.path(root_dir,
                           "analyses/add-histologies/output/ALT PBTA oct 2021 (including all plates)-upd
  # remove existing ones to get newer data
  dplyr::select(-c("OS_days", "OS_status"))
```

```
## Rows: 900 Columns: 115
```

```
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr (56): Kids_First_Biospecimen_ID_DNA, Kids_First_Biospecimen_ID_RNA, Kids...
## dbl (52): TH T/TH N, UBTF Binary, ATRX Reverse Binary, ATRX IHC Binary, ATRX...
## lgl  (7): ATRX Stata, cell_line_composition, DAXX_fusion, ...34, NF...39, CC...
```

```
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
# get survival information
survival_v21 <- readr::read_tsv(file.path(root_dir,
                                   "analyses/add-histologies/input-v21/pbta-histologies.tsv")) %>%
  dplyr::select("Kids_First_Participant_ID", "OS_days", "OS_status", "PFS_days") %>%
  distinct()
```

```
## Rows: 2840 Columns: 38
```

```
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr (33): Kids_First_Biospecimen_ID, sample_id, aliquot_id, Kids_First_Parti...
## dbl  (5): OS_days, age_last_update_days, normal_fraction, tumor_fraction, tu...
```

```
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

Organize data

```r
# join with meta
meta <- meta %>%
  dplyr::left_join(survival_v21) %>%
  dplyr::distinct(Kids_First_Participant_ID, .keep_all = TRUE)
```

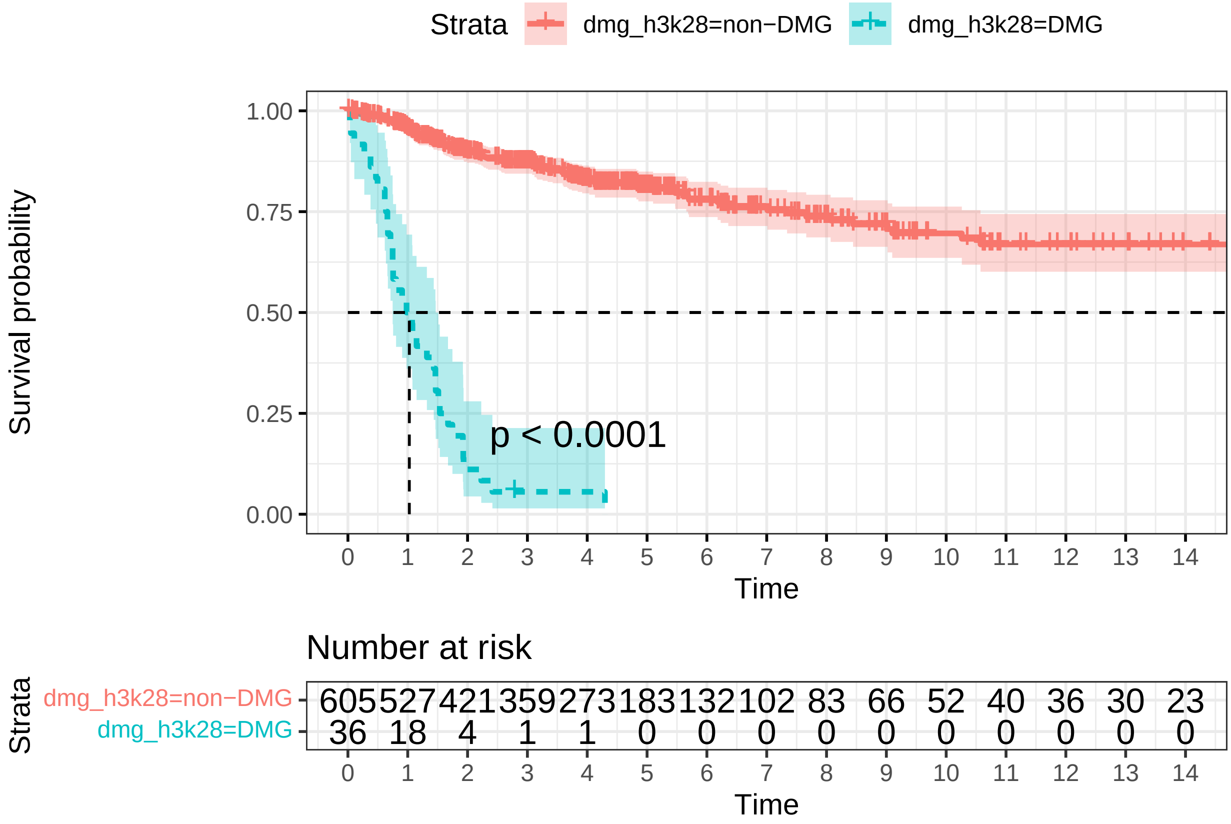
```
## Joining, by = "Kids_First_Participant_ID"
```

```r
# recode for analysis
meta_formatted <- meta %>%
  # recode the categories -
  # DECEASED maps to a survival event status of 1, LIVING maps to a censored observation with value 0
  dplyr::mutate(OS_status_recoded = case_when(
    OS_status == "LIVING" ~ 0,
    OS_status == "DECEASED" ~1
 )) %>%
  # retain only ones with OS days
  dplyr::filter(!is.na(OS_days)) %>%
  # calculte the years
  dplyr::mutate(OS_years = OS_days / 365.25) %>%
  dplyr::mutate(PFS_status = if_else(PFS_days < OS_days, 1, 0)) %>%
  # categorize by telhunt scores
  dplyr::mutate(tel_hunt_cat = case_when(
    `TH T/TH N` > 1.07 ~ "High",
    `TH T/TH N` < 1.07 ~ "Low"
  )) %>%
  # categorize by DMG, H3K28 or not
  dplyr::mutate(dmg_h3k28 = case_when(
    grepl("DMG, H3 K28", molecular_subtype) ~ "DMG",
    TRUE ~ "non-DMG"
  )) %>%
  # categorize ATRX
  dplyr::mutate(atrx_mut = case_when(
    !is.na(`ATRX Mutation`) ~ "ATRX_mut",
    TRUE ~ "non_ATRX_mut"
  )) %>%
  # rename telhunt score and CCA
  dplyr::mutate(telhunt_score = `TH T/TH N`)

# define as factor
meta_formatted$dmg_h3k28 <- factor(meta_formatted$dmg_h3k28, levels = c("non-DMG", "DMG"))
meta_formatted$tel_hunt_cat <- factor(meta_formatted$tel_hunt_cat, levels = c("Low", "High"))
meta_formatted$group <- factor(meta_formatted$group, levels = c("non-HGAT", "HGAT"))
meta_formatted$phenotype <- factor(meta_formatted$phenotype, levels = c("non-ALT", "ALT"))

# define as numberic
meta_formatted$telhunt_score <- as.numeric(meta_formatted$telhunt_score)
meta_formatted_hgat <- meta_formatted %>%
  dplyr::filter(group == "HGAT")
```

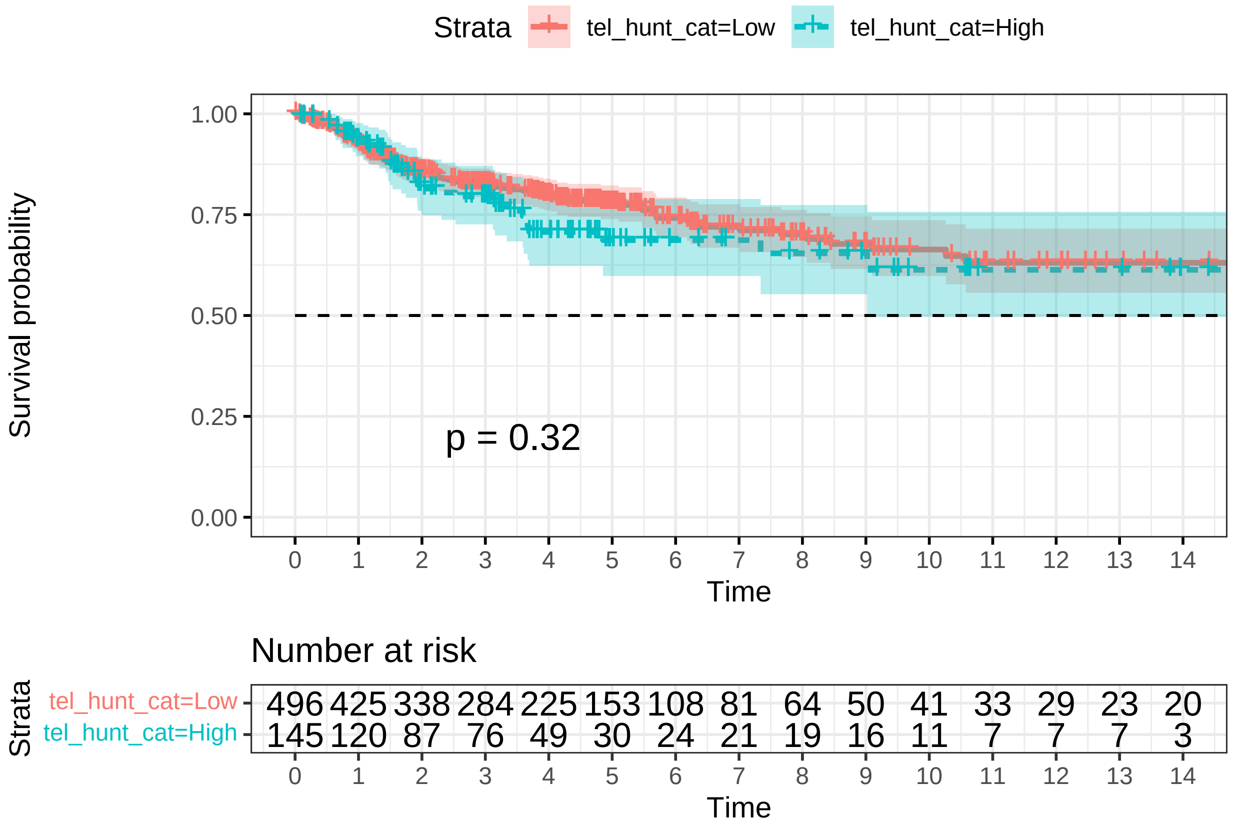
Log Rank analysis

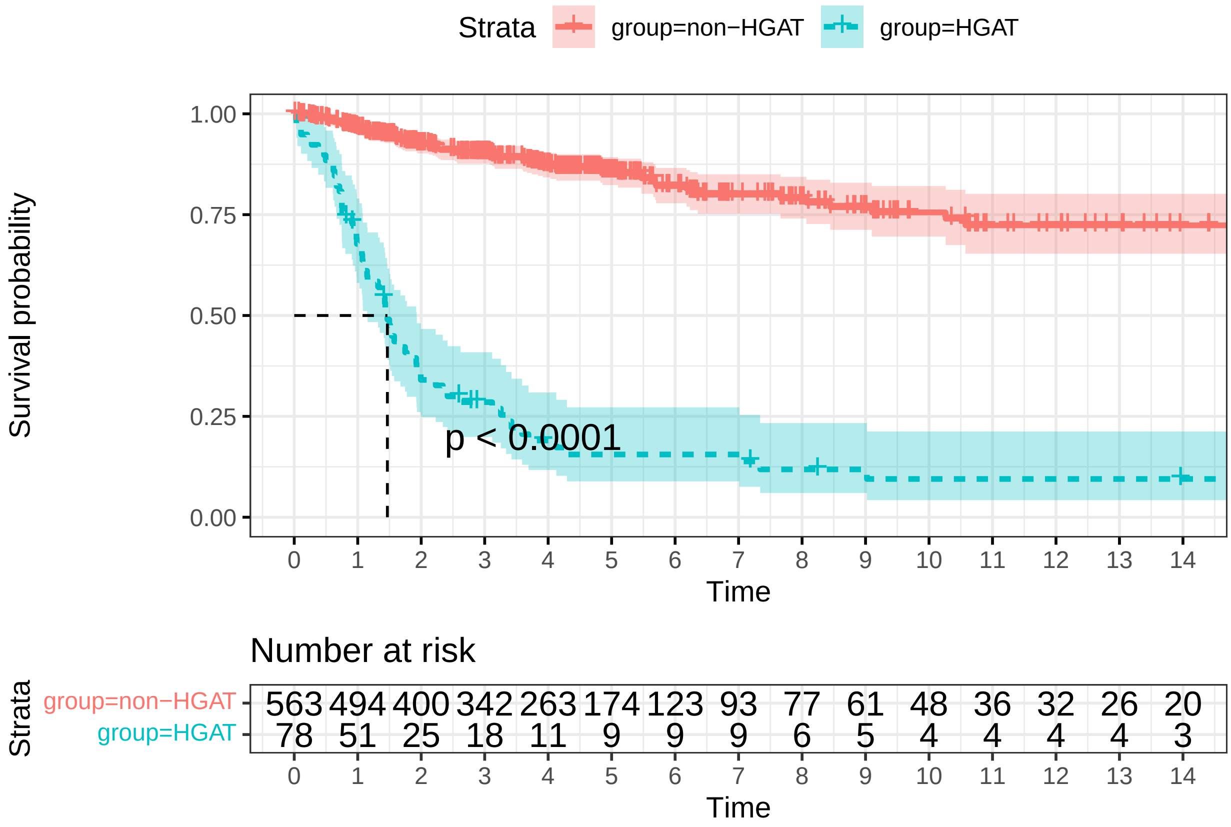
Generate output for categorical files - only hgg group is used

```
for(ind_var in c("dmg_h3k28", "tel_hunt_cat", "group", "atrx_mut", "phenotype")){
  # define model
  model <- paste0("survival::Surv(time = OS_years, event = OS_status_recoded) ~ ", ind_var)

  # depending on which variables are used, data used will be different
  data_used <- meta_formatted

  # run survival analysis
  fit <- survival::survdiff(formula(model),
                            data = data_used)
  # Obtain p value for Chi-Squared stat
  fit$p.value <- pchisq(fit$chisq, df = length(fit$n) - 1, lower = FALSE)

  # save the output
  saveRDS(fit, file.path(output_dir, paste0("log_rank_survival_per_", ind_var, ".RDS")))

  # generate plots fit
  fit_plot <- survfit(formula(model), data = data_used)

  # output the plot
  plot_logrank <- survminer::ggsurvplot(fit_plot,
                                        data=data_used,
                                        xlim = c(0, 14),
                                        break.time.by = 1,
                                        pval = TRUE,
                                        conf.int = TRUE,
                                        risk.table = TRUE, # Add risk table
                                        linetype = "strata", # Change line type by groups
                                        surv.median.line = "hv", # Specify median survival
                                        ggtheme = theme_bw())

  print(plot_logrank)
  # Make this plot a combined plot
  surv_plot_logrank <- cowplot::plot_grid(plot_logrank[[1]],
                                          plot_logrank[[2]],
                                          nrow = 2,
                                          rel_heights = c(2.5, 1))
  # Save the plot
  cowplot::save_plot(filename = file.path(plots_dir,
                                          paste0("logrank_survival_by_", ind_var, ".png")),
                     plot = surv_plot_logrank)


}
```

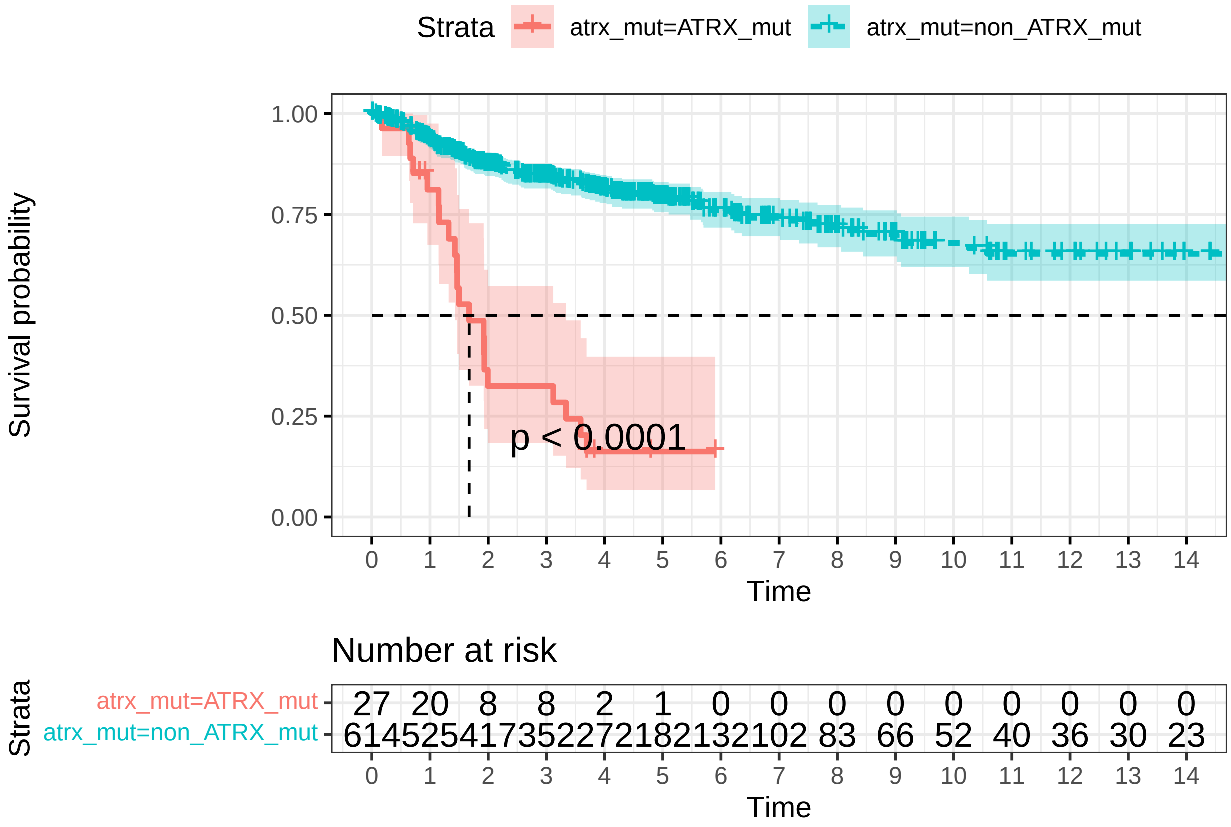


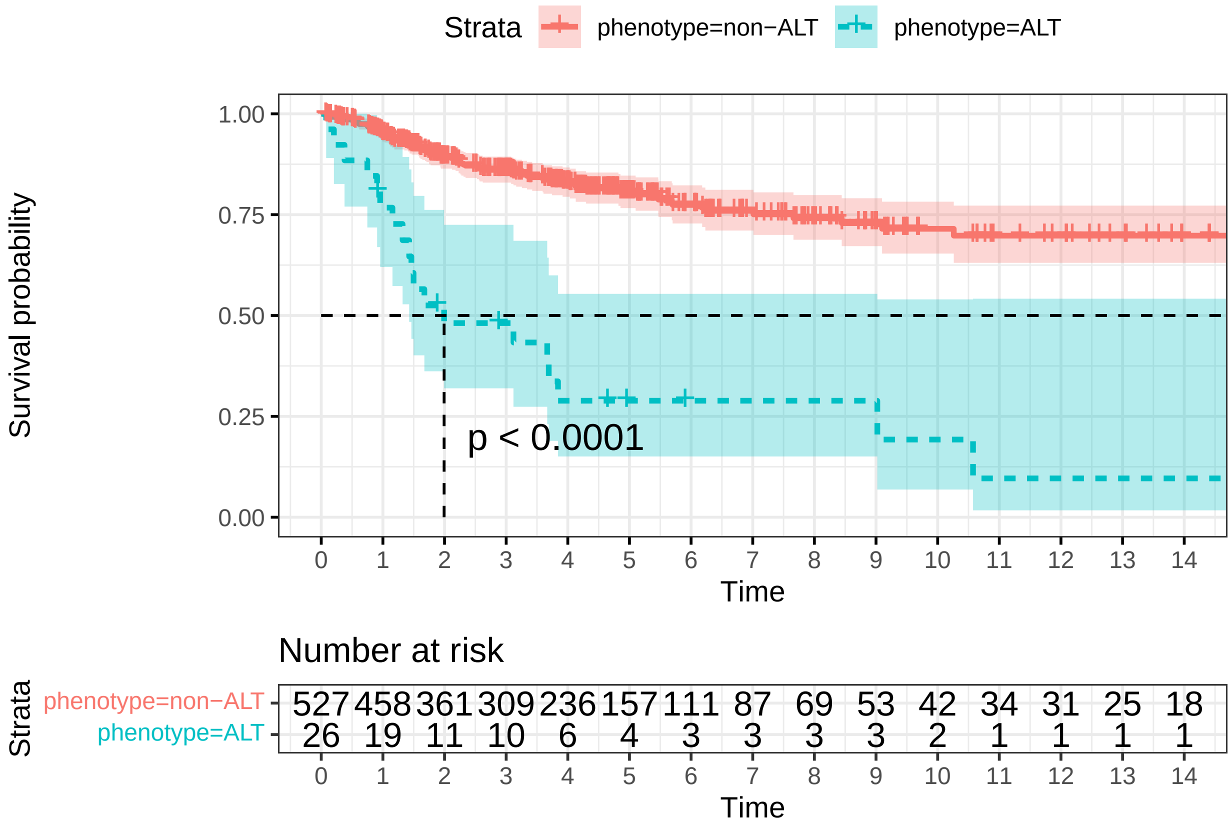
Strata	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
dmg_h3k28=non−DMG	605	527	421	359	273	183	132	102	83	66	52	40	36	30	23
dmg_h3k28=DMG	36	18	4	1	1	0	0	0	0	0	0	0	0	0	0


Strata
tel_hunt_cat=Low
tel_hunt_cat=High
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.32
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time
Number at risk
Strata
tel_hunt_cat=Low 496 425 338 284 225 153 108 81 64 50 41 33 29 23 20
tel_hunt_cat=High 145 120 87 76 49 30 24 21 19 16 11 7 7 7 3
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time



Strata	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
group=non−HGAT	563	494	400	342	263	174	123	93	77	61	48	36	32	26	20
group=HGAT	78	51	25	18	11	9	9	9	6	5	4	4	4	4	3


Strata
atrx_mut=ATRX_mut
atrx_mut=non_ATRX_mut
Survival probability
1.00
0.75
0.50
0.25
0.00
p < 0.0001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time
Number at risk
Strata
atrx_mut=ATRX_mut 27 20 8 8 2 1 0 0 0 0 0 0 0 0 0
atrx_mut=non_ATRX_mut 614 525 417 352 272 182 132 102 83 66 52 40 36 30 23
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time



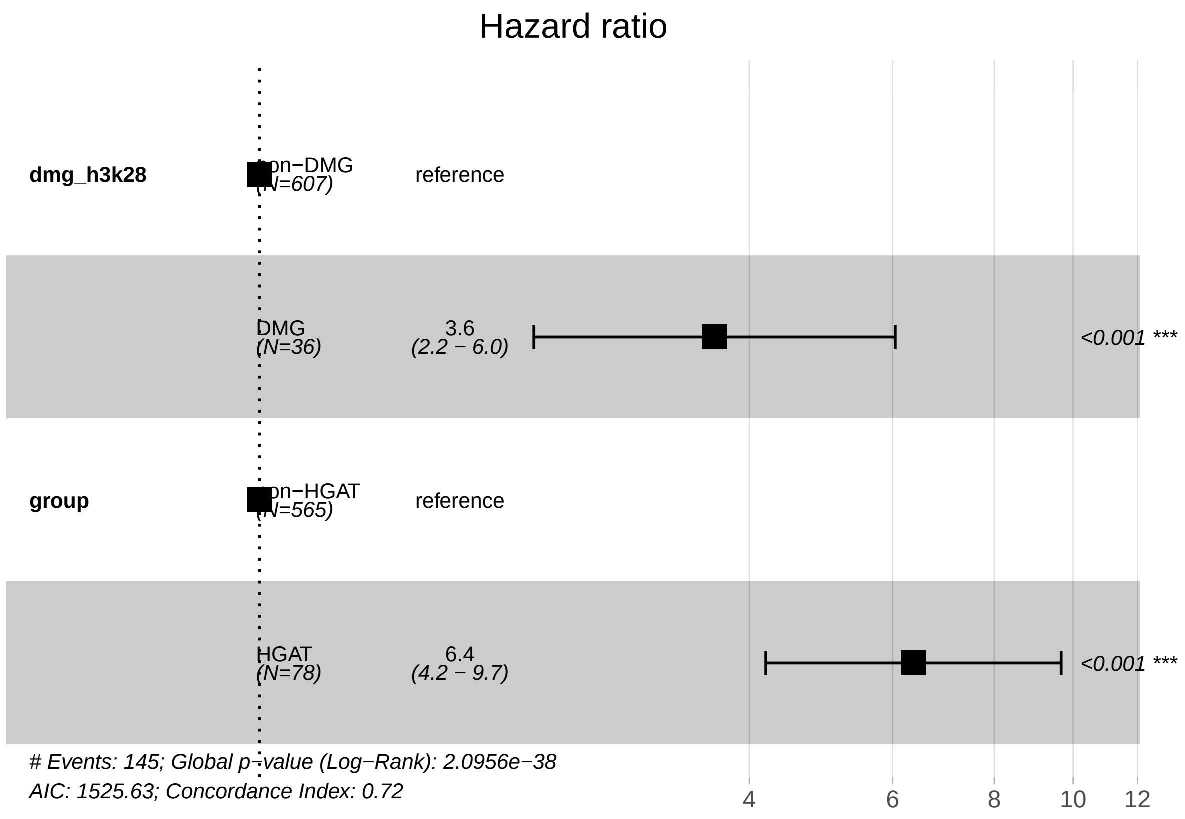
Multivariate analysis

Two comparisons made are `tp53_score + telomerase_score + hgg_group` and `tp53_score + telomerase_score + broad_histology_display`

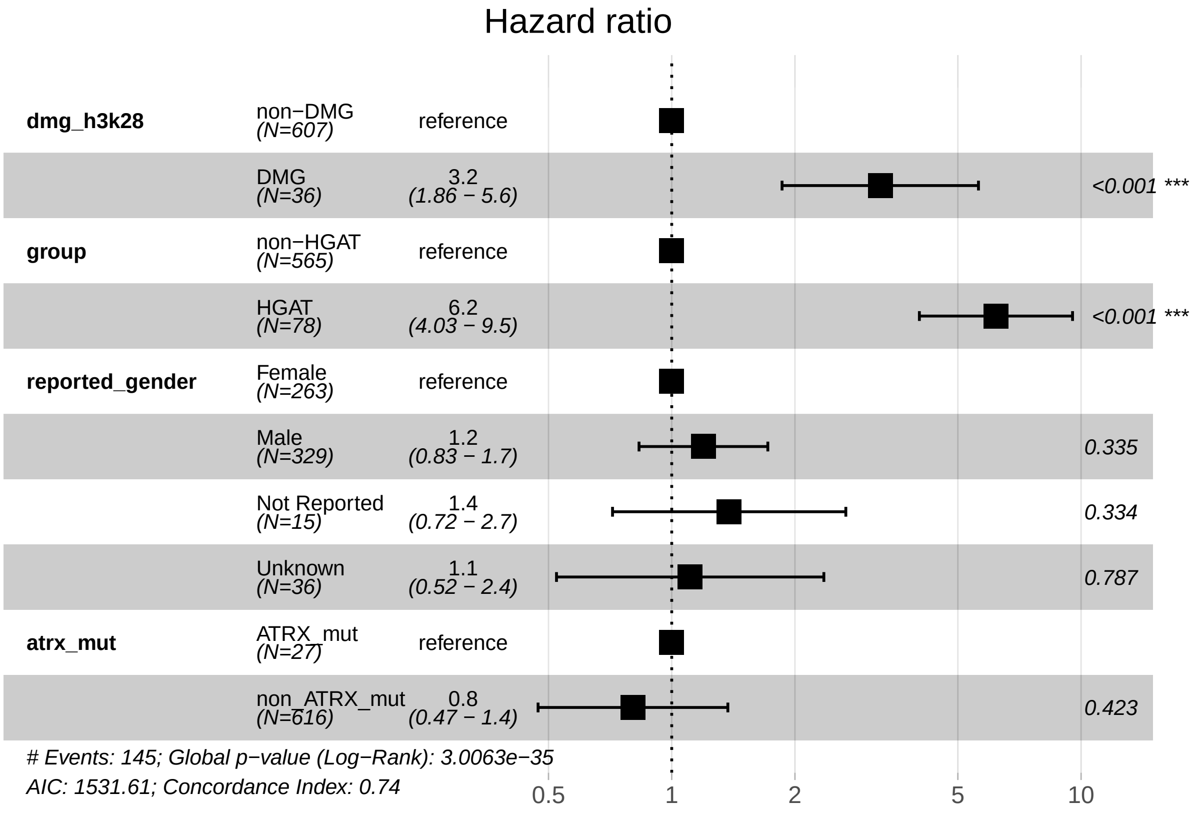
Multivariate analysis - DMG H3K28 vs rest + ALT vs. non-ALT - DMG H3K28 vs rest + ALT vs. non-ALT + sex + ATRX (mut N/Y) - DMG H3K28 vs rest + Telhunt score (categorical) + sex + ATRX (mut N/Y) - DMG H3K28 vs rest + Telhunt score (continuous) + sex + ATRX (mut N/Y)

```
# define multi-variates that we are using for analyzing survival
list_of_variates <- c("dmg_h3k28+group",
                      "dmg_h3k28+group+reported_gender+atrx_mut",
                      "dmg_h3k28+group+tel_hunt_cat+reported_gender+atrx_mut",
                      "dmg_h3k28+group+telhunt_score+reported_gender+atrx_mut",
                      "dmg_h3k28+group+phenotype+reported_gender+atrx_mut"
                      )

# define model
for (ind_var in list_of_variates){
  model <- paste0("survival::Surv(time = OS_years, event = OS_status_recoded) ~ ", ind_var)

  # depending on which variables are used, data used will be different
  data_used <- meta_formatted

  fit <- survival::coxph(
        formula(model),
        data = data_used
      )
  # generate output
  table <- broom::tidy(fit)

  # Save the table data in a TSV
  readr::write_tsv(table, file.path(output_dir, paste0("cox_reg_results_per_", ind_var, ".tsv")))

  print(table)

  # printout the plot
  forest_coxph <- survminer::ggforest(fit, data = data_used)
  print(forest_coxph)

}
```

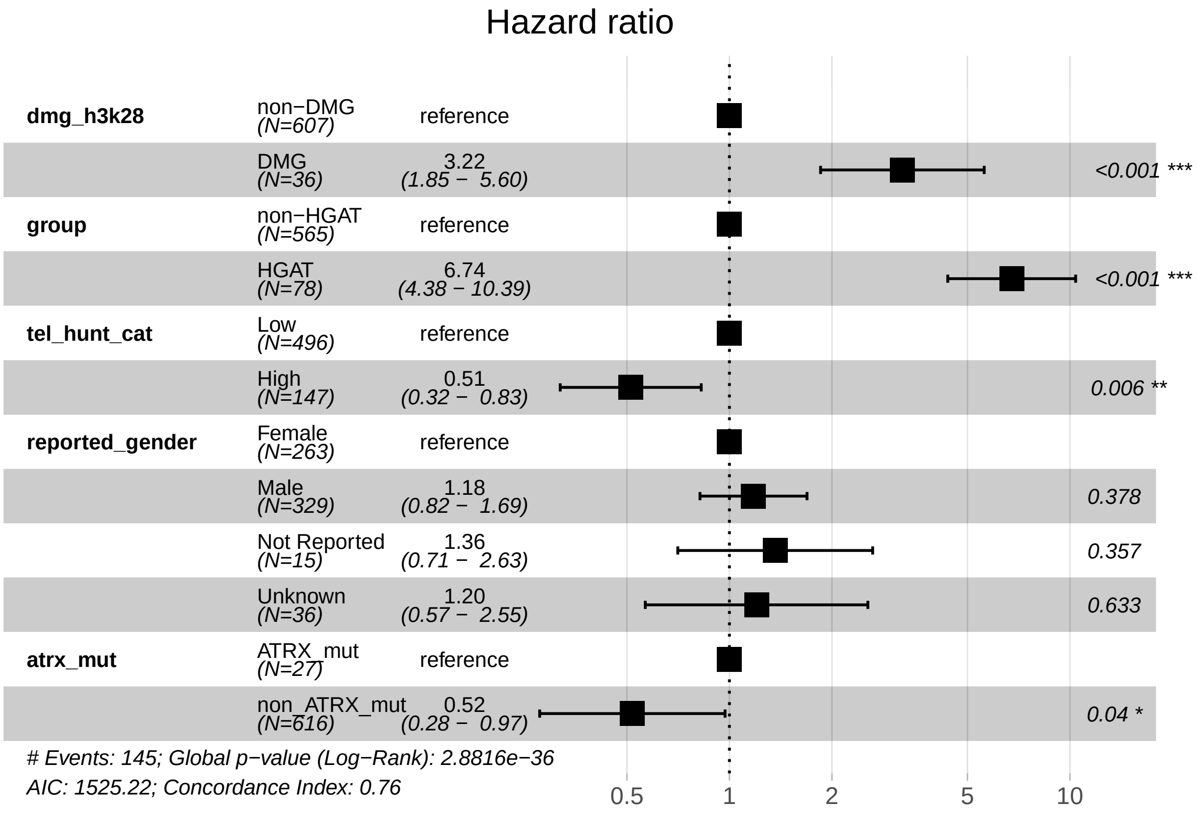
```
## # A tibble: 2 x 5
##   term         estimate std.error statistic  p.value
##   <chr>           <dbl>     <dbl>     <dbl>    <dbl>
## 1 dmg_h3k28DMG     1.29     0.261      4.94 7.93e- 7
## 2 groupHGAT        1.85     0.213      8.68 3.91e-18
```



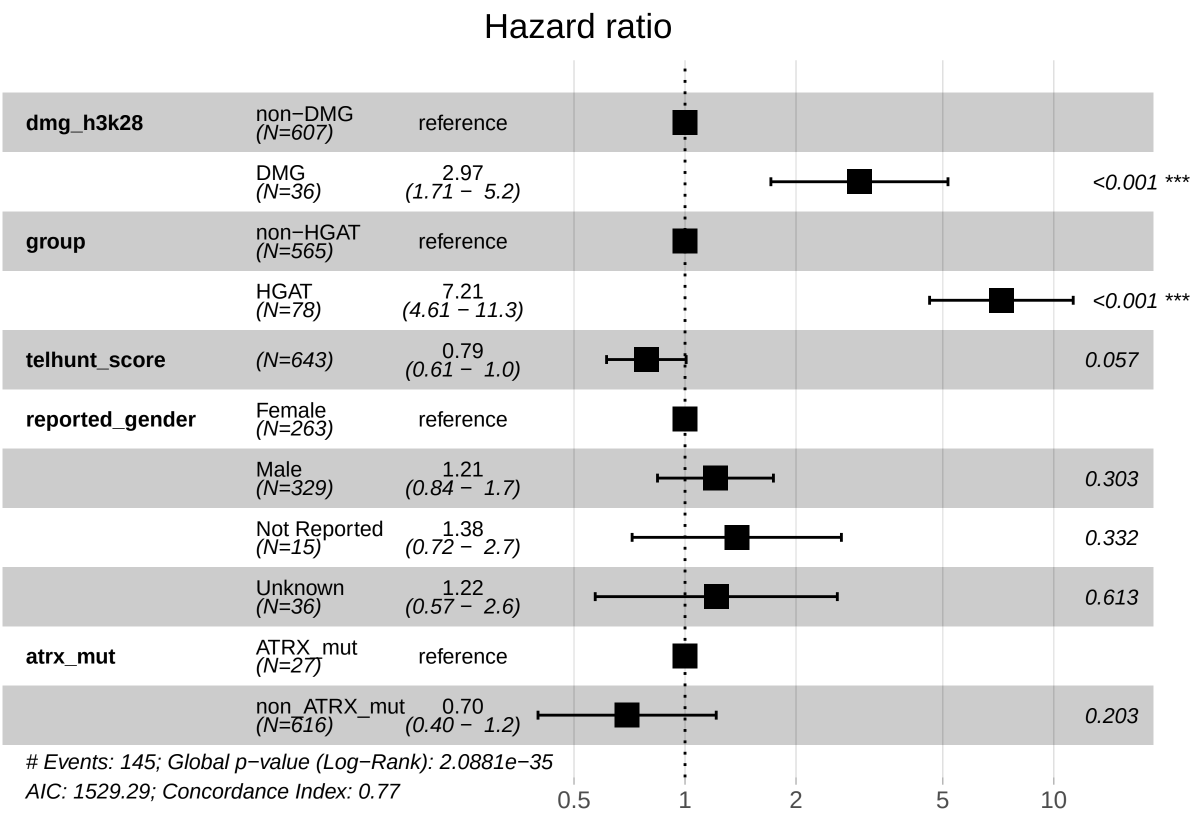
```
## # A tibble: 6 x 5
##   term                         estimate std.error statistic  p.value
##   <chr>                           <dbl>     <dbl>     <dbl>    <dbl>
## 1 dmg_h3k28DMG                    1.17      0.282     4.16  3.17e- 5
## 2 groupHGAT                       1.82      0.220     8.30  1.07e-16
## 3 reported_genderMale             0.178     0.185     0.965 3.35e- 1
## 4 reported_genderNot Reported     0.323     0.335     0.965 3.34e- 1
## 5 reported_genderUnknown          0.104     0.384     0.270 7.87e- 1
## 6 atrx_mutnon_ATRX_mut           -0.218     0.272    -0.801 4.23e- 1
```



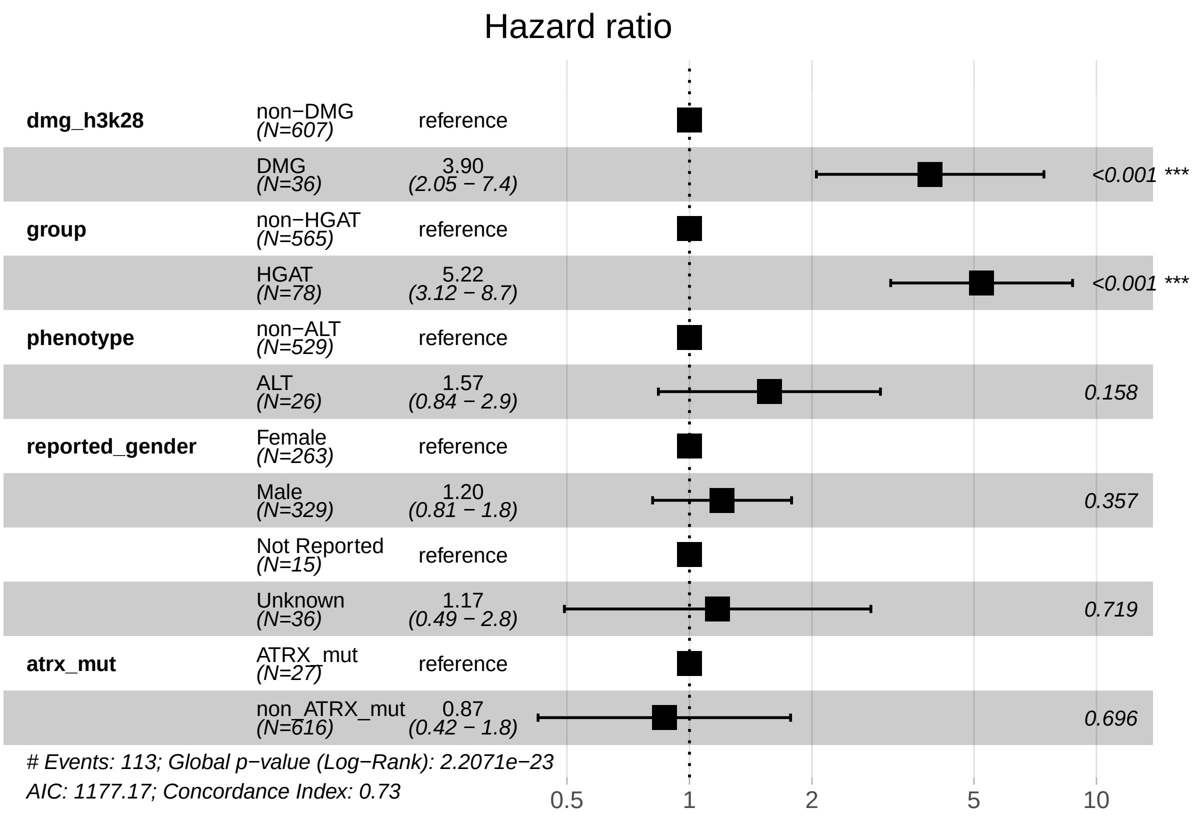
```
## # A tibble: 7 x 5
##   term                         estimate std.error statistic  p.value
##   <chr>                           <dbl>     <dbl>     <dbl>    <dbl>
## 1 dmg_h3k28DMG                    1.17      0.282     4.14  3.43e- 5
## 2 groupHGAT                       1.91      0.221     8.65  5.08e-18
## 3 tel_hunt_catHigh               -0.668     0.243    -2.74  6.06e- 3
## 4 reported_genderMale             0.163     0.185     0.882 3.78e- 1
## 5 reported_genderNot Reported     0.310     0.336     0.921 3.57e- 1
## 6 reported_genderUnknown          0.184     0.384     0.478 6.33e- 1
## 7 atrx_mutnon_ATRX_mut           -0.656     0.320    -2.05  4.01e- 2
```



```
## # A tibble: 7 x 5
##   term                         estimate std.error statistic  p.value
##   <chr>                           <dbl>     <dbl>     <dbl>    <dbl>
## 1 dmg_h3k28DMG                    1.09      0.282     3.86  1.13e- 4
## 2 groupHGAT                       1.98      0.229     8.64  5.86e-18
## 3 telhunt_score                  -0.241     0.127    -1.90  5.68e- 2
## 4 reported_genderMale             0.190     0.185     1.03  3.03e- 1
## 5 reported_genderNot Reported     0.323     0.333     0.969 3.32e- 1
## 6 reported_genderUnknown          0.195     0.386     0.506 6.13e- 1
## 7 atrx_mutnon_ATRX_mut           -0.362     0.284    -1.27  2.03e- 1
```



```
## # A tibble: 6 x 5
##   term                     estimate std.error statistic  p.value
##   <chr>                       <dbl>     <dbl>     <dbl>    <dbl>
## 1 dmg_h3k28DMG                1.36      0.328     4.15  3.38e- 5
## 2 groupHGAT                   1.65      0.262     6.30  3.04e-10
## 3 phenotypeALT                0.452     0.320     1.41  1.58e- 1
## 4 reported_genderMale         0.185     0.201     0.920 3.57e- 1
## 5 reported_genderUnknown      0.159     0.442     0.360 7.19e- 1
## 6 atrx_mutnon_ATRX_mut       -0.142     0.364    -0.390 6.96e- 1
```


Hazard ratio
dmg_h3k28
non−DMG (N=607) reference
DMG (N=36) 3.90 (2.05 − 7.4) <0.001 ***
group
non−HGAT (N=565) reference
HGAT (N=78) 5.22 (3.12 − 8.7) <0.001 ***
phenotype
non−ALT (N=529) reference
ALT (N=26) 1.57 (0.84 − 2.9) 0.158
reported_gender
Female (N=263) reference
Male (N=329) 1.20 (0.81 − 1.8) 0.357
Not Reported (N=15) reference
Unknown (N=36) 1.17 (0.49 − 2.8) 0.719
atrx_mut
ATRX_mut (N=27) reference
non_ATRX_mut (N=616) 0.87 (0.42 − 1.8) 0.696
# Events: 113; Global p−value (Log−Rank): 2.2071e−23
AIC: 1177.17; Concordance Index: 0.73
0.5
1
2
5
10