Targeted exome–based predictors of patterns of progression of colorectal liver metastasis after percutaneous thermal ablation

Statistical Analysis

Iwan Paolucci, PhD*

07 October 2022

Contents

*Department of Interventional Radiology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

1 Setup

```
library(tidyverse)
library(config)
library(readr)
library(dplyr)
library(survival)
library(survminer)
library(stringr)
library(parameters)
library(Rfast)
library(ggpubr)
library(tidyr)
library(forestmodel)
library(ggsci)
library(data.table)
library(gtsummary)
library(gt)
source("R/write_params_to_csv.cox.R")
source("R/finegray_model.R")
options(scipen = 999, digits = 3)
```

2 Prepare Data

2.1 Download data

Download data from RedCAP API. Code omitted.

2.2 Load data

```
data_raw <- read_csv("data/data.csv")
```

2.3 Filter

Remove excluded cases.

```
data <- data_raw %>% filter(date_of_ablation >= "2011-01-01" & !(exclude_reason %in% c(6, 9)))
```

226 patients with initial CLM ablation between 01/01/2011 - 03/30/2022.

```
data %>% select(exclude_reason) %>%
  mutate(exclude_reason = factor(exclude_reason,
                                 labels =
                                   c("< 1 Year imaging follow-up",
                                     "Non-thermal ablation modality (e.g. IRE, Cryo)",
                                     "Genomic mutation profile missing",
                                     "< 46 genes tested",
                                     "Combination with Surgery, TACE or other LRT"))) %>%
  tbl_summary(label = list(exclude_reason = 'Reason for exclusion'))
```

Characteristic	**N = 226**
Reason for exclusion	
< 1 Year imaging follow-up	23 (29%)
Non-thermal ablation modality (e.g. IRE, Cryo)	7 (8.8%)
Genomic mutation profile missing	41 (51%)
< 46 genes tested	4 (5.0%)
Combination with Surgery, TACE or other LRT	5 (6.2%)
Unknown	146

```
data <- data %>% filter(exclude_reason %in% c(NA, 3, 4))

data$mutation.analysis <- data$exclude_reason %in% c(NA) & !is.na(data$apc)

data %>% select(mutation.analysis) %>%
  tbl_summary(label = list(mutation.analysis = 'Included for mutation analysis'))
```

Characteristic	**N = 191**
Included for mutation analysis	101 (53%)

Total 191 included for progression pattern analysis and 101 for mutation analysis.

2.4 Preprocess

```
data <- within(data, {
    deceased = deceased == 1
    gt_46_tested <- data$nr_tested_genes >= 46
    nr_tested_genes = ifelse(is.na(nr_tested_genes), 0, nr_tested_genes)
    a0_ablation = a0_ablation == 1
    n_positive = n_positive == 1
    max_diameter = max_diameter / 10.0
    repeat_lrt = repeat_lrt == 1
    prior_resection = prior_resection == 1
    os_m = overall_survival / 30.25
    metastasis = factor(metastasys_sync,
                        levels = c("sync", "meta"),
                        labels = c("Synchronous", "Metachronous"))
})

data <- within(data, {
    ablation_modality = as.factor(ablation_modality)
    cohort = factor(cohort, labels = c("2005-2015", "2015-2020"))
    year_num = as.numeric(format(data$date_of_ablation, '%Y'))
    year = as.factor(format(data$date_of_ablation, '%Y'))
})

data <- within(data, {
    ltp = ifelse(intrahepatic_recurrence == FALSE, FALSE, local_recurrence == 1)
    ltpfs = ifelse(ltp, time_to_lc, time_to_lfu)
    ihp_outside = ifelse(intrahepatic_recurrence == FALSE, FALSE, new_intrahepatic_lesions == 1)
    ihpfs_outside = ifelse(ihp_outside, time_to_new_lesions, time_to_lfu)
    repeat_lrt = ifelse(intrahepatic_recurrence, repeat_lrt, FALSE)
    ihp_any = ltp | ihp_outside
    ihpfs_any = ifelse(ihp_any, ifelse(ltpfs < ihpfs_outside, ltpfs, ihpfs_outside), time_to_lfu)
})

data$os_ihp = data$overall_survival - data$ihpfs_any
data$os_ihp_m = data$os_ihp / 30.25


data <- within(data, {
  ehp = extrahepatic_recurrence == 1
  ehpfs = ifelse(ehp, time_to_ehp, time_to_lfu)
  ehm = ehm == 1
})

data$any_progression <- data$ltp | data$ihp_outside | data$ehp

data <- within(data, {
    chemo_yes_no = chemo_yes_no == 1
    chemo_lines = ifelse(chemo_lines == "NAVU", NA, as.numeric(chemo_lines))
})

tmp = list(primary_side = factor(data$primary_location_detail))
```

```
levels(tmp$primary_side) <- list(
  Right = c("ce","ac", "hf"),
  Left =  c("dc","r","rs", "sc","sf"),
  Transverse = c("tc")
)
data$primary_side <- tmp$primary_side

data <- within(data, {
  right_side = primary_side == "Right"
  ehp_before = ifelse(ehp,
                      ifelse(ihp_any, ehpfs <= ihpfs_outside & ehpfs <= ltpfs, TRUE), FALSE)
  time_to_lfu_m = time_to_lfu / 30.25
  time_to_lfu_y = time_to_lfu / 365
  ehp_m = ehp / 30.25
  ihpfs_outside_m = ihpfs_outside / 30.25
  ltpfs = ltpfs / 30.25
  ehpfs_before = ifelse(ehp_before, ehpfs, ihpfs_any)
})
```

2.5 Extract mutations

```
cols.p53.logic <- c("mdm2","mdm4", "cdkn2a", "tp53", "atm")
data[cols.p53.logic] <- sapply(data[cols.p53.logic], as.logical)


cols.tgfb.logic <- c("smad4")
data[cols.tgfb.logic] <- sapply(data[cols.tgfb.logic], as.logical)

cols.wnt.logic <- c("rnf43", "apc", "ctnnb1")
data[cols.wnt.logic] <- sapply(data[cols.wnt.logic], as.logical)

cols.ras.logic <- c("egfr", "erbb2", "erbb3", "erbb4", "met", "pdgfra", "fgfr1",
                    "fgfr2", "fgfr3", "fgfr4", "kit", "igf1r", "ret", "ros1", "alk",
                    "flt3", "ntrk1", "jak2", "cbl", "abl1", "nf1", "ptpn11",
                    "kras", "nras", "hras", "araf", "braf", "raf1", "rac1",
                    "mapk1", "map2k1", "map2k2", "ntrk2", "ntrk3", "crebbp")
data[cols.ras.logic] <- sapply(data[cols.ras.logic], as.logical)

cols.pi3k.logic <- c("pik3ca", "pten", "pik3cb", "pik3r1", "akt1", "akt2", "akt3",
                     "ppp2r1a", "stk11", "tsc1", "tsc2", "rheb", "rictor", "mtor")
data[cols.pi3k.logic] <- sapply(data[cols.pi3k.logic], as.logical)

cols.wnt.logic <- c("rnf43", "apc", "ctnnb1")
data[cols.wnt.logic] <- sapply(data[cols.wnt.logic], as.logical)

cols.notch.logic <- c("fbxw7", "notch1", "notch2", "notch3")
data[cols.notch.logic] <- sapply(data[cols.notch.logic], as.logical)

cols.cell.logic <- c("cdkn1b", "cdkn2b", "ccne1", "rb1", "ccnd1", "ccnd2", "ccnd3",
                     "cdk2", "cdk4", "cdk6")
data[cols.cell.logic] <- sapply(data[cols.cell.logic], as.logical)
```

2.5.1 Grouping KRAS, NRAS, BRAF into one category RAS/BRAF

```
data$rasbraf <- data$kras | data$nras | data$braf
```

2.5.2 Grouping pathways

```
data <- data %>%
    rowwise() %>%
    mutate(
        p53 = any(mdm2, mdm4, cdkn2a, tp53, atm, na.rm = TRUE),
        tgfb = any(smad4, na.rm = TRUE),
        wnt = any(rnf43, apc, ctnnb1, na.rm = TRUE),
        ras = any(egfr, erbb2, erbb3, erbb4, met, pdgfra, fgfr1, fgfr2, fgfr3,
                  fgfr4, kit, igf1r, ret, ros1, alk,
                  flt3, ntrk1, jak2, cbl, abl1, nf1, ptpn11, kras, hras, nras,
                  araf, braf, raf1, rac1, mapk1, map2k1,
```

```
                  map2k2, ntrk2, ntrk3, crebbp, na.rm = TRUE),
        pi3k = any(pik3ca, pten, akt1, pik3cb, pik3r1, akt2, akt3, ppp2r1a, stk11,
                   tsc1, tsc2, rheb, rictor, mtor, na.rm = TRUE),
        notch = any(fbxw7, notch1, notch2, notch3, na.rm=TRUE),
        cell = any(cdkn1b, cdkn2b, cdkn2a, ccne1, rb1, ccnd1, ccnd2, ccnd3, cdk2,
                   cdk4, cdk6, na.rm = TRUE)
        )
data$wnt <- ifelse(is.na(data$apc), NA, data$wnt)
data <- within(data, {
  num_altered_paths = (p53 + tgfb + ras + pi3k + notch + cell + wnt)
})
```

3 Population

3.1 Baseline characteristics

```
data.tbl <- data %>%
  haven::as_factor() %>%
  mutate(chemo_lines = as.factor(chemo_lines)) %>%
  select(age_at_procedure, sex, primary_location, right_side, metastasis, n_positive,
         prior_resection, ehm, nr_of_tumors, max_diameter, time_to_lfu_m,
         chemo_yes_no, chemo_lines,chemo_regimen___5fu1, chemo_regimen___ox,
         chemo_regimen___iri, chemo_regimen___bev) %>%
  tbl_summary(
    label = list(age_at_procedure ~ "Age",
                 sex ~ "Sex",
                 primary_location ~ "Primary location",
                 right_side ~ "Right-sided primary tumor",
                 metastasis ~ "Metastasis",
                 n_positive ~ "Positive lymphnodes",
                 prior_resection ~ "Prior resection",
                 ehm ~ "Extrahepatic metastasis",
                 chemo_yes_no ~ "Pre-ablation chemotherapy",
                 chemo_lines ~ "Lines of pre-ablation chemotherapy",
                 chemo_regimen___5fu1 ~ "Fluorouracil-based regimen",
                 chemo_regimen___ox ~ "Oxaliplatin",
                 chemo_regimen___iri ~ "Irinotecan",
                 chemo_regimen___bev ~ "Use of Bevacizumab",
                 nr_of_tumors ~ "Nr of ablated CLM",
                 max_diameter ~ "Max CLM diameter",
                 time_to_lfu_m ~ "Time to last FU"),
    type = list(nr_of_tumors ~ 'continuous'),
    statistic = list(all_continuous() ~ "{median} [{p25}, {p75}]"),
    digits = list(all_continuous() ~ 2,
                  all_categorical() ~ 1),
    missing_text = "N/A") %>%
  bold_labels()


data.tbl
```

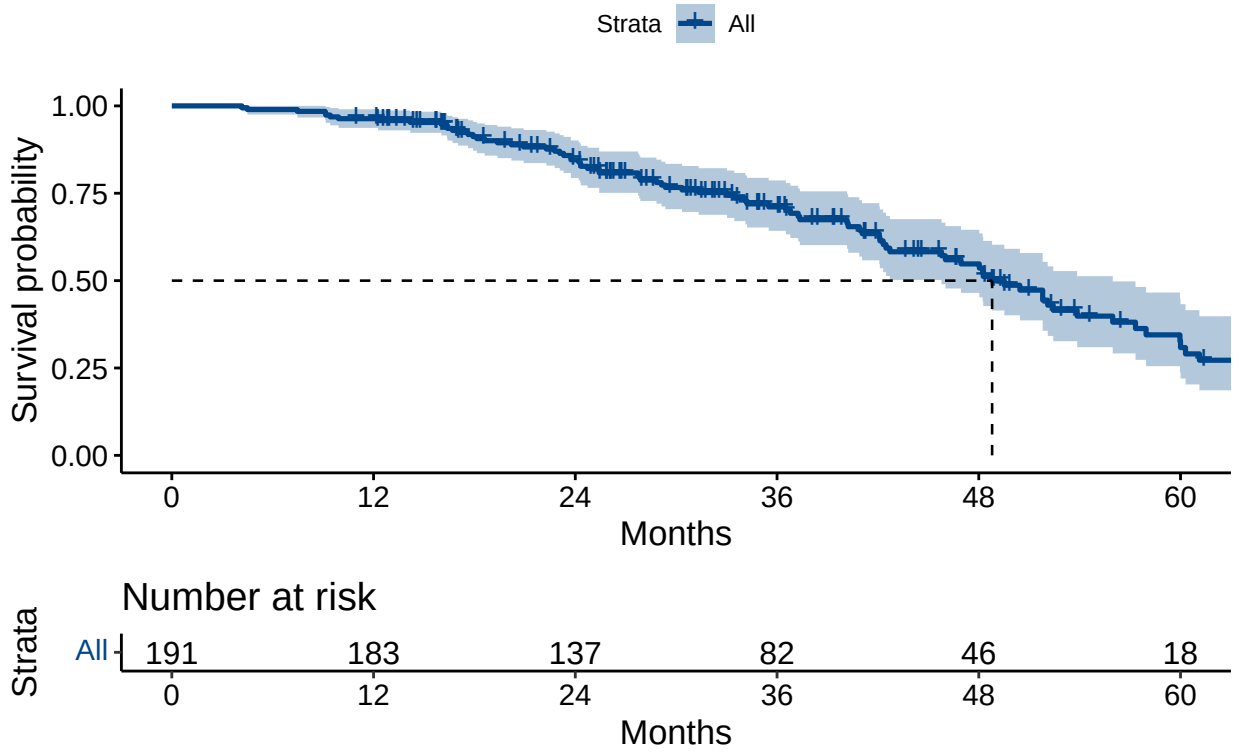
Characteristic	**N = 191**
Age	56.70 [48.60, 66.55]
Sex	
f	70.0 (36.6%)
m	121.0 (63.4%)
Primary location	
colon	152.0 (79.6%)
rectum	39.0 (20.4%)
Right-sided primary tumor	45.0 (23.6%)
Metastasis	
Synchronous	102.0 (54.3%)
Metachronous	86.0 (45.7%)

Characteristic	N = 191
N/A	3
Positive lymphnodes	114.0 (59.7%)
Prior resection	117.0 (61.3%)
Extrahepatic metastasis	111.0 (58.1%)
Nr of ablated CLM	1.00 [1.00, 2.00]
Max CLM diameter	1.40 [1.00, 2.00]
Time to last FU	31.50 [18.46, 44.26]
Pre-ablation chemotherapy	142.0 (74.3%)
Lines of pre-ablation chemotherapy	
1	83.0 (43.5%)
2	54.0 (28.3%)
3	5.0 (2.6%)
0	49.0 (25.7%)
Fluorouracil-based regimen	127.0 (66.5%)
Oxaliplatin	74.0 (38.7%)
Irinotecan	70.0 (36.6%)
Use of Bevacizumab	90.0 (47.1%)

```
data.tbl %>% as_gt() %>% gtsave(filename = "out/population.rtf")
```

3.2 Baseline survival

```
data$clm <- TRUE

ggsurvplot(survfit(Surv(os_m, deceased) ~ 1, data = data),
           data = data,
           risk.table = TRUE, tables.height = 0.25,
           surv.median.line = 'hv',
           xlim = c(0, 60), break.time.by = 12, xlab = "Months",
           palette = "lancet")
```


Strata
All
1.00
0.75
0.50
0.25
0.00
Survival probability
0
12
24
36
48
60
Months

Number at risk
Strata
All
191
183
137
82
46
18
0
12
24
36
48
60
Months

3.3 Overview mutations

```
mutations.tbl <- data %>%
  filter(mutation.analysis) %>%
  haven::as_factor() %>%
  select(p53, tp53, ras, rasbraf, wnt, apc, tgfb, smad4, pi3k, pik3ca,
         notch, fbxw7, cell, rb1) %>%
  tbl_summary(
    digits = list(all_categorical() ~ 1),
    missing_text = "N/A") %>%
  bold_labels()

mutations.tbl
```

Characteristic	N = 101
p53	75.0 (74.3%)
tp53	75.0 (74.3%)
ras	48.0 (47.5%)
rasbraf	42.0 (41.6%)
wnt	64.0 (63.4%)
apc	61.0 (60.4%)
tgfb	16.0 (15.8%)
smad4	16.0 (15.8%)
pi3k	17.0 (16.8%)
pik3ca	12.0 (11.9%)
notch	5.0 (5.0%)
fbxw7	5.0 (5.0%)
cell	4.0 (4.0%)
rb1	4.0 (4.0%)

Exclude RB1 and Cell Cycle pathway from further analysis

3.4 Type of progression

```
data$multiple_progression <- apply(data[,c('ihp_outside', 'ltp', 'ehp')], 1, sum) > 1
data$ihp_both <- data$ltp & data$ihp_outside
data$no_progression <- !(data$ltp | data$ihp_outside | data$ehp)

progression.tbl <- data %>%
  select(ltp, ihp_outside, ihp_both, ihp_any, ehp, no_progression,
         multiple_progression, repeat_lrt, deceased) %>%
  tbl_summary(
    by = deceased,
    label = list(
      ltp = 'Local tumor progression',
      ihp_outside = "Intrahepatic progression outside the ablation zone",
      ihp_both = "Local and outside intrahepatic progression",
      ihp_any = 'Any kind of intrahepatic progression',
      ehp = 'Extrahepatic progression',
      no_progression = 'No progression',
      multiple_progression = 'Multiple sites of progression',
      repeat_lrt = 'Repeat LRT'
      ),
    digits = list(all_categorical() ~ 1)
  ) %>%
  add_overall() %>%
  add_p() %>%
  bold_labels()

progression.tbl
```

Characteristic	**Overall**, N = 191	**FALSE**, N = 109	**TRUE**, N = 82	**p-value**
Local tumor progression	43.0 (22.5%)	19.0 (17.4%)	24.0 (29.3%)	0.053
Intrahepatic progression outside the ablation zone	121.0 (63.4%)	62.0 (56.9%)	59.0 (72.0%)	0.032
Local and outside intrahepatic progression	29.0 (15.2%)	11.0 (10.1%)	18.0 (22.0%)	0.024
Any kind of intrahepatic progression	135.0 (70.7%)	70.0 (64.2%)	65.0 (79.3%)	0.024
Extrahepatic progression	143.0 (74.9%)	72.0 (66.1%)	71.0 (86.6%)	0.001
No progression	26.0 (13.6%)	20.0 (18.3%)	6.0 (7.3%)	0.028
Multiple sites of progression	115.0 (60.2%)	54.0 (49.5%)	61.0 (74.4%)	<0.001
Repeat LRT	71.0 (37.2%)	48.0 (44.0%)	23.0 (28.0%)	0.024

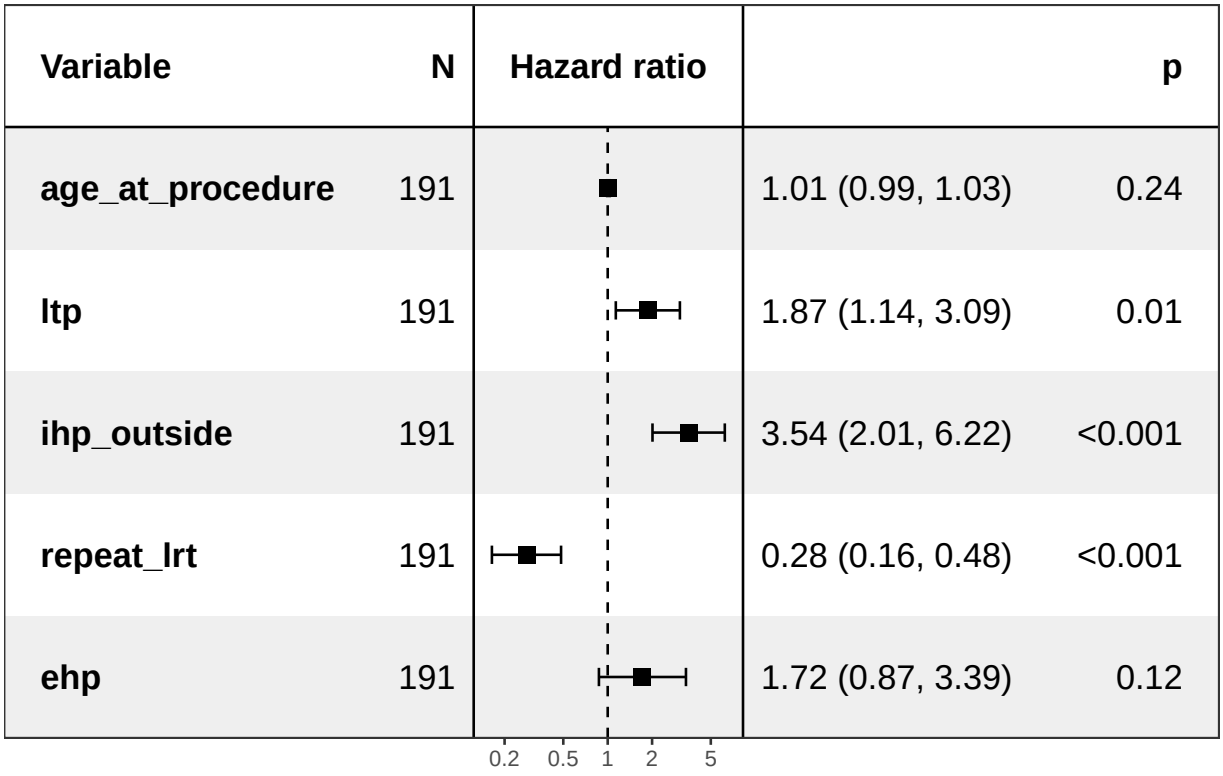
4 Overall survival

```
model.format <- forest_model_format_options(point_size = 3)
```

4.1 By progression pattern

Not adjusted

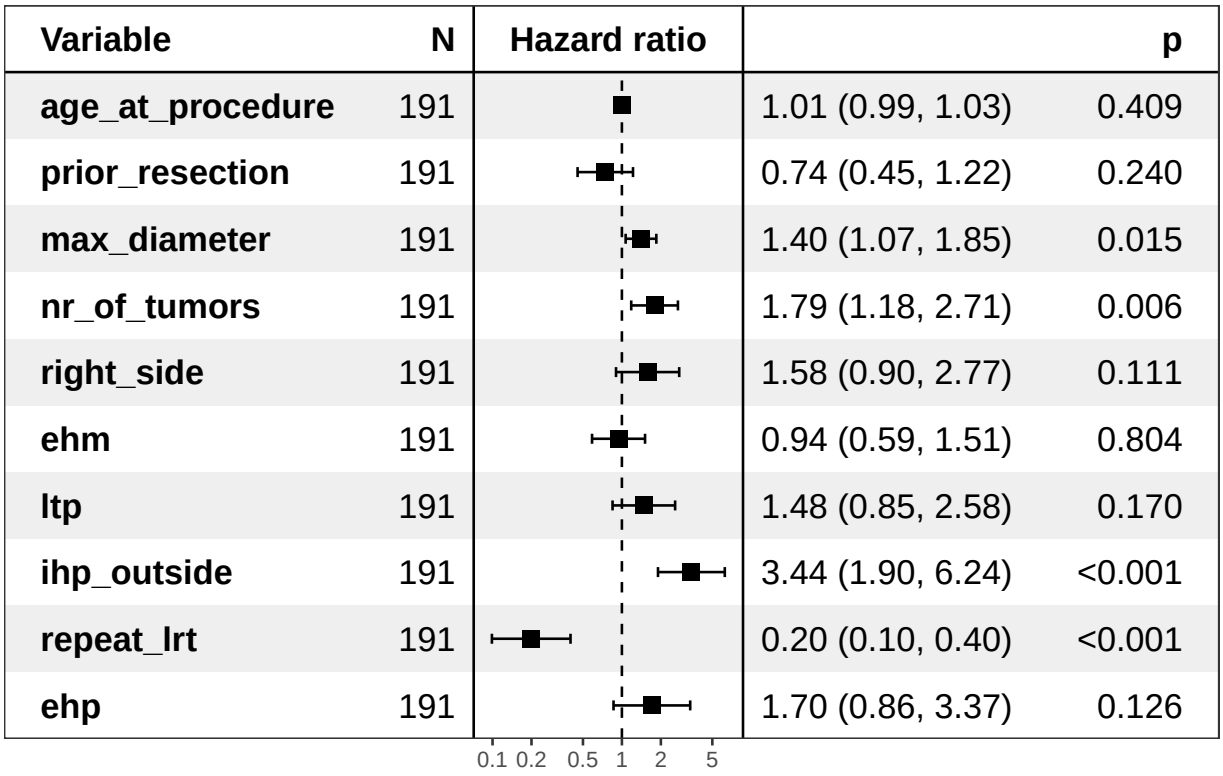
```
os.multivariable <- coxph(Surv(os_m, deceased) ~  age_at_procedure +
                            ltp + ihp_outside + repeat_lrt + ehp,
                  data = data)

forest_model(model = os.multivariable,
             exponentiate = TRUE,
             exclude_infinite_cis = TRUE,
             format_options = model.format)
```




```
os.multivariable %>% tbl_regression(
  exponentiate = TRUE,
  show_single_row = all_categorical()
)
```

Characteristic	HR	95% CI	p-value
age_at_procedure	1.01	0.99, 1.03	0.2
ltp	1.87	1.14, 3.09	0.014
ihp_outside	3.54	2.01, 6.22	<0.001
repeat_lrt	0.28	0.16, 0.48	<0.001
ehp	1.72	0.87, 3.39	0.12

```
data$no_progression <- !data$any_progression

os.multivariable <- coxph(Surv(os_m, deceased) ~  age_at_procedure + prior_resection +
                            max_diameter + nr_of_tumors + right_side + ehm +
                             ltp + ihp_outside + repeat_lrt + ehp,
                  data = data)

forest_model(model = os.multivariable,
             exponentiate = TRUE,
             exclude_infinite_cis = TRUE,
             format_options = model.format)
```




```
os.multi.tbl <- os.multivariable %>% tbl_regression(
  label = list(age_at_procedure ~ "Age",
               prior_resection ~ "Prior resection",
               max_diameter ~ "Diameter of largest CLM",
               nr_of_tumors ~ "Number of ablated CLM",
               right_side ~ "Right side primary",
               ehm ~ "Presence of extrahepatic metastasis",
```

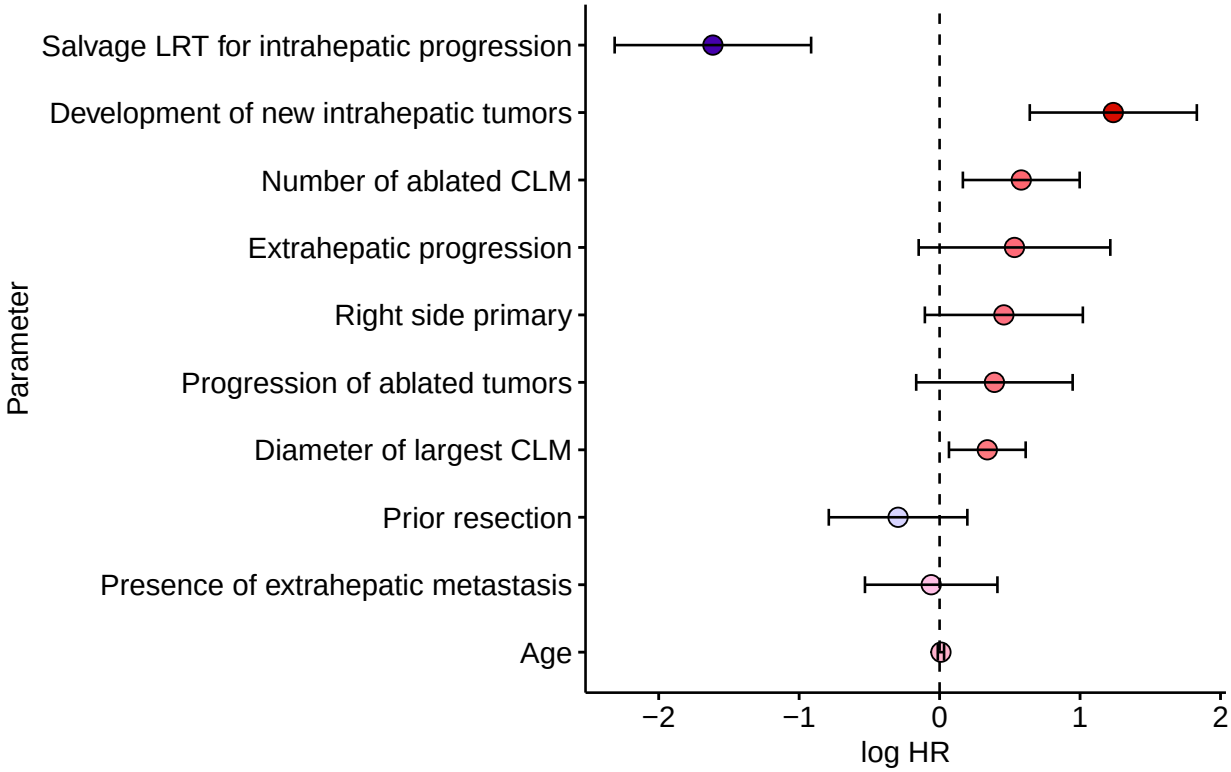
```
               ltp ~ "Progression of ablated tumors",
               ihp_outside ~ "Development of new intrahepatic tumors",
               repeat_lrt ~ "Salvage LRT for intrahepatic progression",
               ehp ~ "Extrahepatic progression"),
  exponentiate = TRUE,
  show_single_row = all_categorical(),
  pvalue_fun = function(x) style_pvalue(x, digits = 2),
  estimate_fun = function(x) style_ratio(x, digits = 2)
  )

os.multi.tbl
```

Characteristic	**HR**	**95% CI**	**p-value**
Age	1.01	0.99, 1.03	0.41
Prior resection	0.74	0.45, 1.22	0.24
Diameter of largest CLM	1.40	1.07, 1.85	0.015
Number of ablated CLM	1.79	1.18, 2.71	0.006
Right side primary	1.58	0.90, 2.77	0.11
Presence of extrahepatic metastasis	0.94	0.59, 1.51	0.80
Progression of ablated tumors	1.48	0.85, 2.58	0.17
Development of new intrahepatic tumors	3.44	1.90, 6.24	<0.001
Salvage LRT for intrahepatic progression	0.20	0.10, 0.40	<0.001
Extrahepatic progression	1.70	0.86, 3.37	0.13

```
os.multi.tbl %>% as_gt() %>% gtsave(filename = "out/os.rtf")
```

```
source("R/my_forest_plot.R")
```

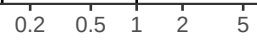
```
os.multi.tbl$table_body %>%
  select(var_label, estimate, conf.low, conf.high) %>%
  mutate(estimate = log(estimate),
         conf.low = log(conf.low),
         conf.high = log(conf.high)) %>%
  arrange(abs(estimate)) %>%
  ggdotplot(., x = 'var_label', y = 'estimate',
            fill = "estimate",
            orientation = 'horizontal',
            xlab = "Parameter", ylab = "log HR") +
  scale_fill_gsea() +
  guides(fill="none") +
  geom_errorbar(aes(group = var_label, ymax = conf.high, ymin = conf.low),
                position = position_dodge(width = 0.8), width = 0.25) +
  geom_hline(yintercept = 0, linetype = "dashed")
```


Salvage LRT for intrahepatic progression
Development of new intrahepatic tumors
Number of ablated CLM
Extrahepatic progression
Right side primary
Progression of ablated tumors
Diameter of largest CLM
Prior resection
Presence of extrahepatic metastasis
Age
Parameter
−2
−1
0
1
2
log HR

5 Intrahepatic progression anywhere

```
ihpfs.any <- coxph(Surv(ihpfs_any, ihp_any) ~ age_at_procedure + prior_resection + a0_ablation +
                           max_diameter + nr_of_tumors + ehm + right_side  +
                           p53 + ras + wnt + tgfb + pi3k + notch + cell,
                 data = data)

forest_model(model = ihpfs.any,
             exponentiate = TRUE,
             format_options = model.format)
```

Variable	N	Hazard ratio		p
age_at_procedure	101		1.00 (0.97, 1.02)	0.935
prior_resection	101		1.09 (0.66, 1.80)	0.740
a0_ablation	101		0.42 (0.22, 0.82)	0.010
max_diameter	101		1.20 (0.91, 1.57)	0.192
nr_of_tumors	101		1.42 (0.96, 2.09)	0.077
ehm	101		0.75 (0.43, 1.31)	0.316
right_side	101		0.90 (0.48, 1.66)	0.725
p53	101		0.97 (0.54, 1.75)	0.923
ras	101		0.68 (0.38, 1.20)	0.179
wnt	101		0.56 (0.33, 0.94)	0.030
tgfb	101		2.74 (1.42, 5.27)	0.003
pi3k	101		1.00 (0.51, 1.98)	0.996
notch	101		0.63 (0.17, 2.29)	0.482
cell	101		2.53 (0.78, 8.20)	0.121



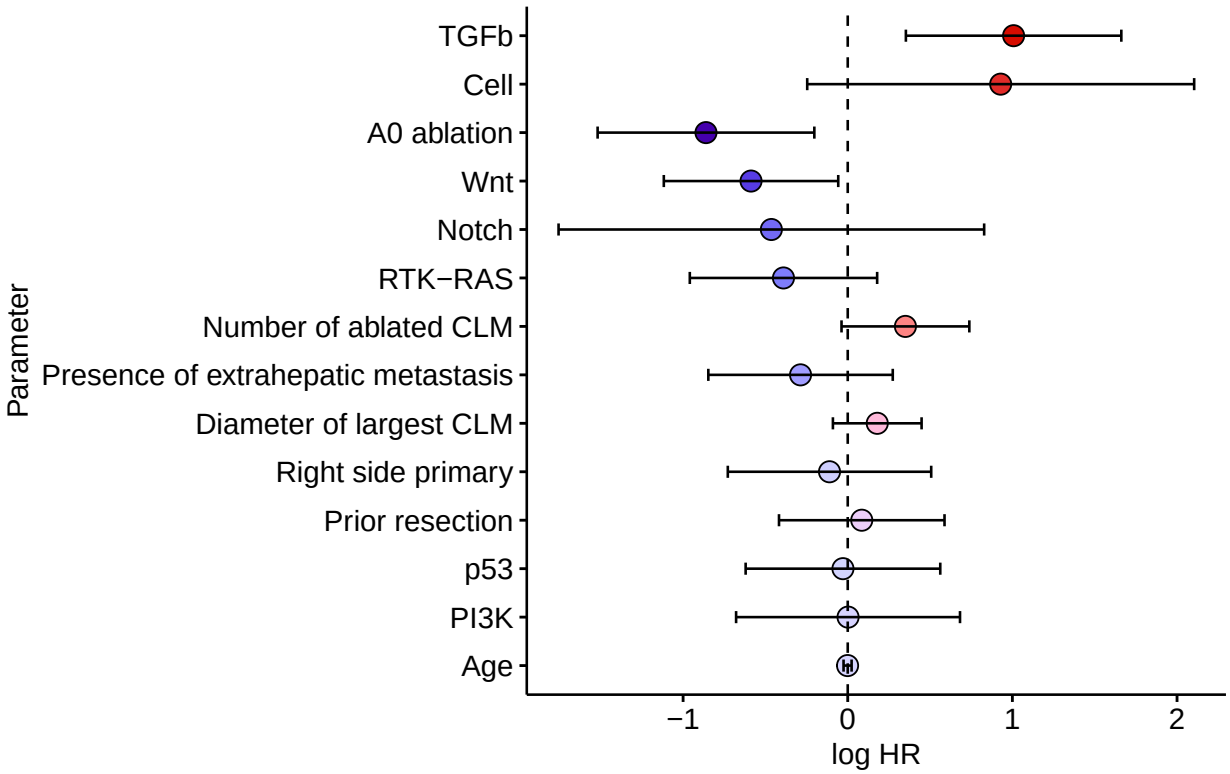

```
ihpfs.any.tbl <- ihpfs.any %>% tbl_regression(
  label = list(age_at_procedure ~ "Age",
               prior_resection ~ "Prior resection",
               a0_ablation ~ "A0 ablation",
               max_diameter ~ "Diameter of largest CLM",
               nr_of_tumors ~ "Number of ablated CLM",
               right_side ~ "Right side primary",
               ehm ~ "Presence of extrahepatic metastasis",
               p53 ~ "p53",
               ras ~ "RTK-RAS",
               wnt ~ "Wnt",
               tgfb ~ "TGFb",
               pi3k ~ "PI3K",
               notch ~ "Notch",
               cell ~ "Cell"),
```

```
  exponentiate = TRUE,
  show_single_row = all_categorical(),
  pvalue_fun = function(x) style_pvalue(x, digits = 2),
  estimate_fun = function(x) style_ratio(x, digits = 2)
)

ihpfs.any.tbl
```

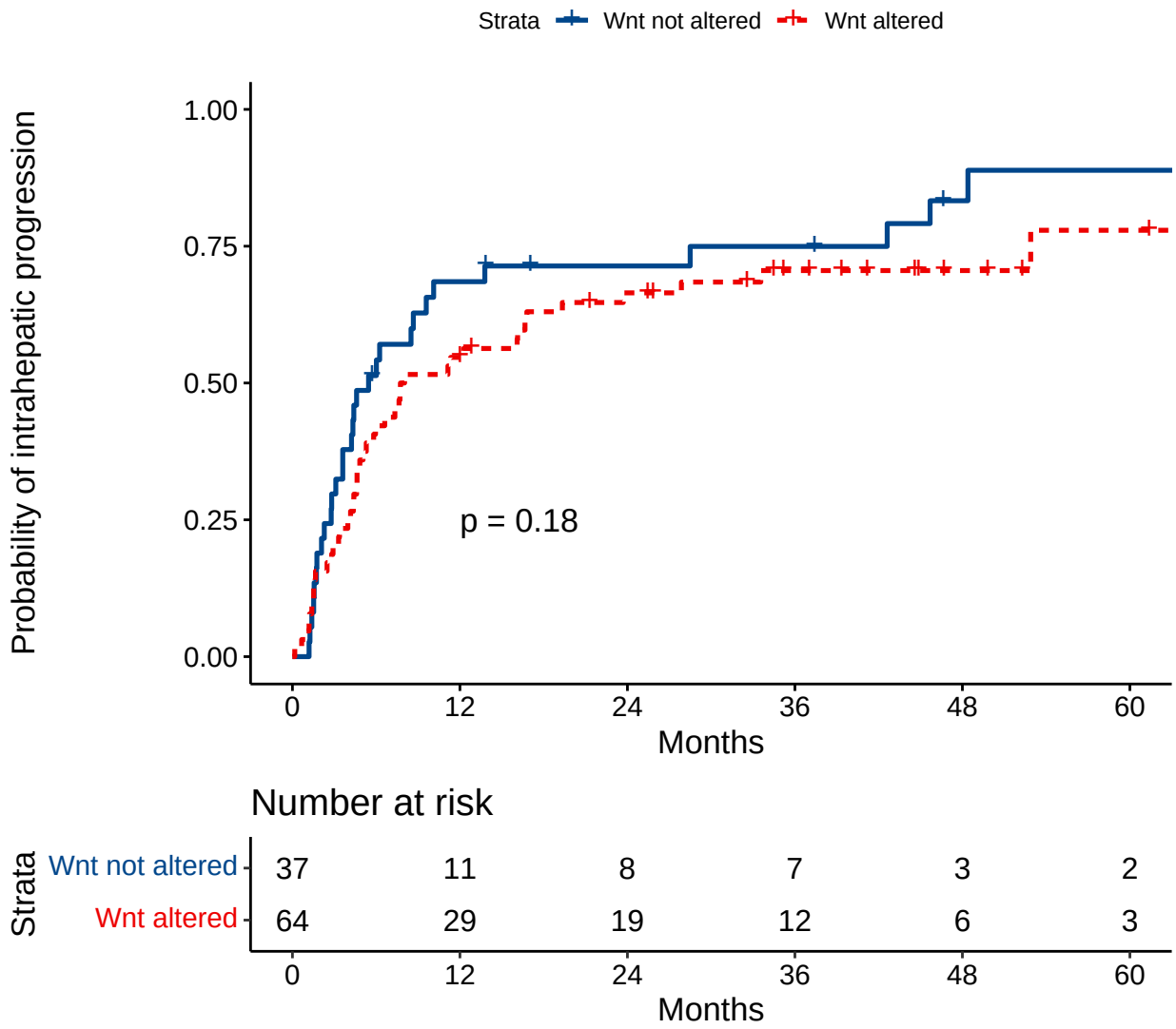
Characteristic	HR	95% CI	p-value
Age	1.00	0.97, 1.02	0.94
Prior resection	1.09	0.66, 1.80	0.74
A0 ablation	0.42	0.22, 0.82	0.010
Diameter of largest CLM	1.20	0.91, 1.57	0.19
Number of ablated CLM	1.42	0.96, 2.09	0.077
Presence of extrahepatic metastasis	0.75	0.43, 1.31	0.32
Right side primary	0.90	0.48, 1.66	0.73
p53	0.97	0.54, 1.75	0.92
RTK-RAS	0.68	0.38, 1.20	0.18
Wnt	0.56	0.33, 0.94	0.030
TGFb	2.74	1.42, 5.27	0.003
PI3K	1.00	0.51, 1.98	>0.99
Notch	0.63	0.17, 2.29	0.48
Cell	2.53	0.78, 8.20	0.12

```
ihpfs.any.tbl %>% as_gt() %>% gtsave(filename = "out/ihpfs_any.rtf")
```

```
ihpfs.any.tbl$table_body %>%
  select(var_label, estimate, conf.low, conf.high) %>%
  mutate(estimate = log(estimate),
         conf.low = log(conf.low),
         conf.high = log(conf.high)) %>%
  arrange(abs(estimate)) %>%
  ggdotplot(., x = 'var_label', y = 'estimate',
            fill = "estimate",
            orientation = 'horizontal',
            xlab = "Parameter", ylab = "log HR") +
  scale_fill_gsea() +
  guides(fill="none") +
  geom_errorbar(aes(group = var_label, ymax = conf.high, ymin = conf.low),
                position = position_dodge(width = 0.8), width = 0.25) +
  geom_hline(yintercept = 0, linetype = "dashed")
```


TGFb
Cell
A0 ablation
Wnt
Notch
RTK−RAS
Number of ablated CLM
Presence of extrahepatic metastasis
Diameter of largest CLM
Right side primary
Prior resection
p53
PI3K
Age
Parameter
−1
0
1
2
log HR

```
ggsurvplot(survfit(Surv(ihpfs_any / 30.25, ihp_any) ~ wnt,
                   data = data[data$mutation.analysis,]),
           pval = TRUE, risk.table = TRUE, conf.int = FALSE,
           pval.coord = c(12, 0.25),
           data = data[data$mutation.analysis,],
           legend.labs = c("Wnt not altered", "Wnt altered"),
           xlim = c(0, 60), break.time.by = 12, xlab = "Months",
           ylim = c(0, 1),
           ylab = "Probability of intrahepatic progression",
           palette = "lancet", fun = "event",
           linetype = c("strata"))
```

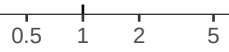


5.1 Intrahepatic progression outside the ablation zone

```
data.ihp <- data %>% filter(ihp_any)
```

```
ihpfs.out <- coxph(Surv(ihpfs_outside, ihp_outside) ~ age_at_procedure + prior_resection +
                             max_diameter + nr_of_tumors + ehm  + a0_ablation + right_side +
                             p53 + ras + wnt + tgfb + pi3k + notch + cell,
                   data = data)

forest_model(model = ihpfs.out,
             exponentiate = TRUE,
             format_options = model.format)
```

Variable	N	Hazard ratio		p
age_at_procedure	101		1.00 (0.97, 1.02)	0.863
prior_resection	101		1.55 (0.90, 2.69)	0.116
max_diameter	101		1.24 (0.93, 1.66)	0.148
nr_of_tumors	101		1.77 (1.20, 2.63)	0.004
ehm	101		0.78 (0.43, 1.42)	0.416
a0_ablation	101		0.61 (0.31, 1.21)	0.154
right_side	101		1.24 (0.65, 2.39)	0.514
p53	101		0.73 (0.39, 1.37)	0.330
ras	101		0.65 (0.35, 1.20)	0.165
wnt	101		0.60 (0.35, 1.03)	0.062
tgfb	101		2.75 (1.39, 5.45)	0.004
pi3k	101		0.82 (0.37, 1.80)	0.620
notch	101		1.10 (0.30, 4.11)	0.882
cell	101		2.12 (0.65, 6.92)	0.212



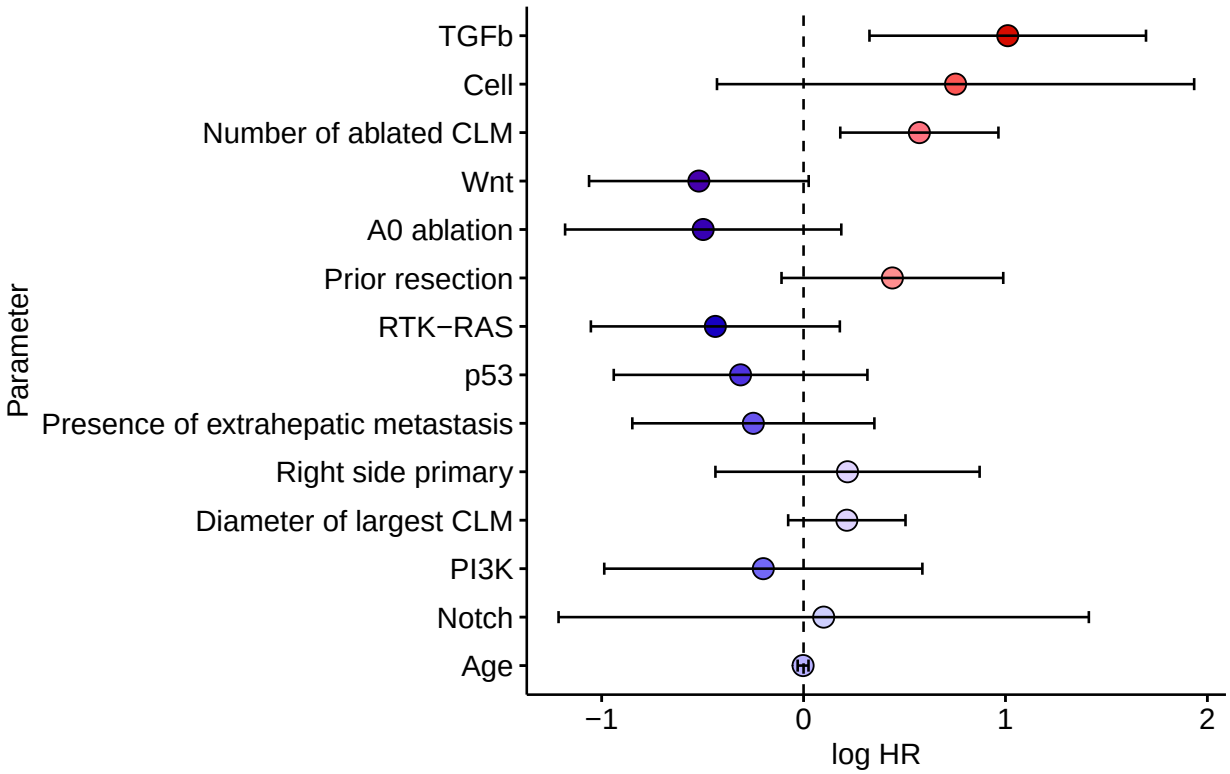

```
ihpfs.tbl <- ihpfs.out %>% tbl_regression(
  label = list(age_at_procedure ~ "Age",
               prior_resection ~ "Prior resection",
               a0_ablation ~ "A0 ablation",
               max_diameter ~ "Diameter of largest CLM",
               nr_of_tumors ~ "Number of ablated CLM",
               right_side ~ "Right side primary",
               ehm ~ "Presence of extrahepatic metastasis",
               p53 ~ "p53",
               ras ~ "RTK-RAS",
               wnt ~ "Wnt",
               tgfb ~ "TGFb",
               pi3k ~ "PI3K",
```

```
               notch ~ "Notch",
               cell ~ "Cell"),
  exponentiate = TRUE,
  show_single_row = all_categorical(),
  pvalue_fun = function(x) style_pvalue(x, digits = 2),
  estimate_fun = function(x) style_ratio(x, digits = 2)
  )
ihpfs.tbl
```

Characteristic	HR	95% CI	p-value
Age	1.00	0.97, 1.02	0.86
Prior resection	1.55	0.90, 2.69	0.12
Diameter of largest CLM	1.24	0.93, 1.66	0.15
Number of ablated CLM	1.77	1.20, 2.63	0.004
Presence of extrahepatic metastasis	0.78	0.43, 1.42	0.42
A0 ablation	0.61	0.31, 1.21	0.15
Right side primary	1.24	0.65, 2.39	0.51
p53	0.73	0.39, 1.37	0.33
RTK-RAS	0.65	0.35, 1.20	0.16
Wnt	0.60	0.35, 1.03	0.062
TGFb	2.75	1.39, 5.45	0.004
PI3K	0.82	0.37, 1.80	0.62
Notch	1.10	0.30, 4.11	0.88
Cell	2.12	0.65, 6.92	0.21

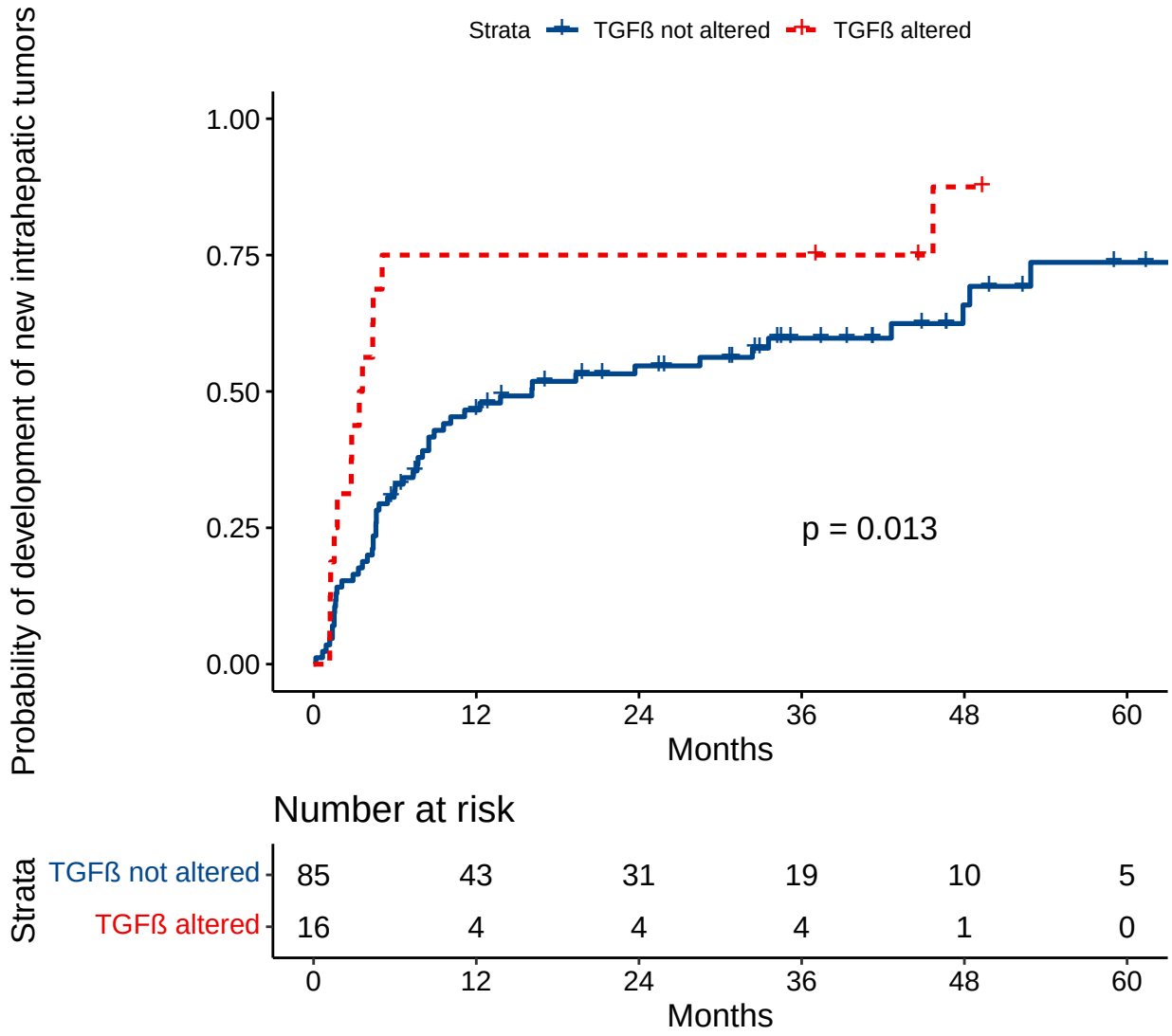
```
ihpfs.tbl %>% as_gt() %>% gtsave(filename = "out/ihpfs.rtf")
```

```
ihpfs.tbl$table_body %>%
  select(var_label, estimate, conf.low, conf.high) %>%
  mutate(estimate = log(estimate),
         conf.low = log(conf.low),
         conf.high = log(conf.high)) %>%
  arrange(abs(estimate)) %>%
  ggdotplot(., x = 'var_label', y = 'estimate',
            fill = "estimate",
            orientation = 'horizontal',
            xlab = "Parameter", ylab = "log HR") +
  scale_fill_gsea() +
  guides(fill="none") +
  geom_errorbar(aes(group = var_label, ymax = conf.high, ymin = conf.low),
                position = position_dodge(width = 0.8), width = 0.25) +
  geom_hline(yintercept = 0, linetype = "dashed")
```


TGFb
Cell
Number of ablated CLM
Wnt
A0 ablation
Prior resection
RTK−RAS
p53
Presence of extrahepatic metastasis
Right side primary
Diameter of largest CLM
PI3K
Notch
Age
Parameter
−1
0
1
2
log HR

5.1.1 Survival curve based on TGFb

```
ggsurvplot(survfit(Surv(ihpfs_outside / 30.25, ihp_outside) ~ tgfb,
                   data = data[data$mutation.analysis, ]),
           pval = TRUE, risk.table = TRUE, conf.int = FALSE,
           pval.coord = c(36, 0.25),
           data = data[data$mutation.analysis, ],
           legend.labs = c("TGF\u03B2 not altered", "TGF\u03B2 altered"),
           xlim = c(0, 60), break.time.by = 12, xlab = "Months",
           ylim = c(0, 1),
           ylab = "Probability of development of new intrahepatic tumors",
           palette = "lancet", fun = "event",
           linetype = c("strata"))
```



```r
ihpfs.tgfb <- survfit(Surv(ihpfs_outside / 30.25, ihp_outside) ~ tgfb,
                     data = data[data$mutation.analysis,])
print(ihpfs.tgfb)
```

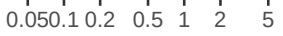
```
## Call: survfit(formula = Surv(ihpfs_outside/30.25, ihp_outside) ~ tgfb,
##     data = data[data$mutation.analysis, ])
##
##              n events median 0.95LCL 0.95UCL
## tgfb=FALSE 85     52  16.10    8.50    47.9
## tgfb=TRUE  16     13   3.49    1.75      NA
```

5.2 Intrahepatic progression at the ablation zone

Notch excluded because it leads to infinite CIs

```
ltp.multi <- coxph(Surv(ltpfs, ltp) ~ age_at_procedure + prior_resection + right_side +
                             max_diameter + nr_of_tumors + ehm + a0_ablation +
                             p53 + ras + wnt + tgfb + pi3k  + cell,
                   data = data)

forest_model(model = ltp.multi,
             exponentiate = TRUE,
             format_options = model.format)
```

Variable	N	Hazard ratio		p
age_at_procedure	101		0.97 (0.94, 1.01)	0.20
prior_resection	101		0.36 (0.16, 0.83)	0.02
right_side	101		0.82 (0.28, 2.34)	0.71
max_diameter	101		1.59 (1.04, 2.42)	0.03
nr_of_tumors	101		1.04 (0.55, 1.98)	0.90
ehm	101		0.56 (0.25, 1.28)	0.17
a0_ablation	101		0.17 (0.05, 0.66)	0.01
p53	101		1.42 (0.50, 4.05)	0.51
ras	101		0.75 (0.32, 1.77)	0.52
wnt	101		0.83 (0.36, 1.93)	0.66
tgfb	101		1.46 (0.47, 4.54)	0.51
pi3k	101		1.03 (0.36, 2.99)	0.95
cell	101		1.04 (0.12, 9.25)	0.97



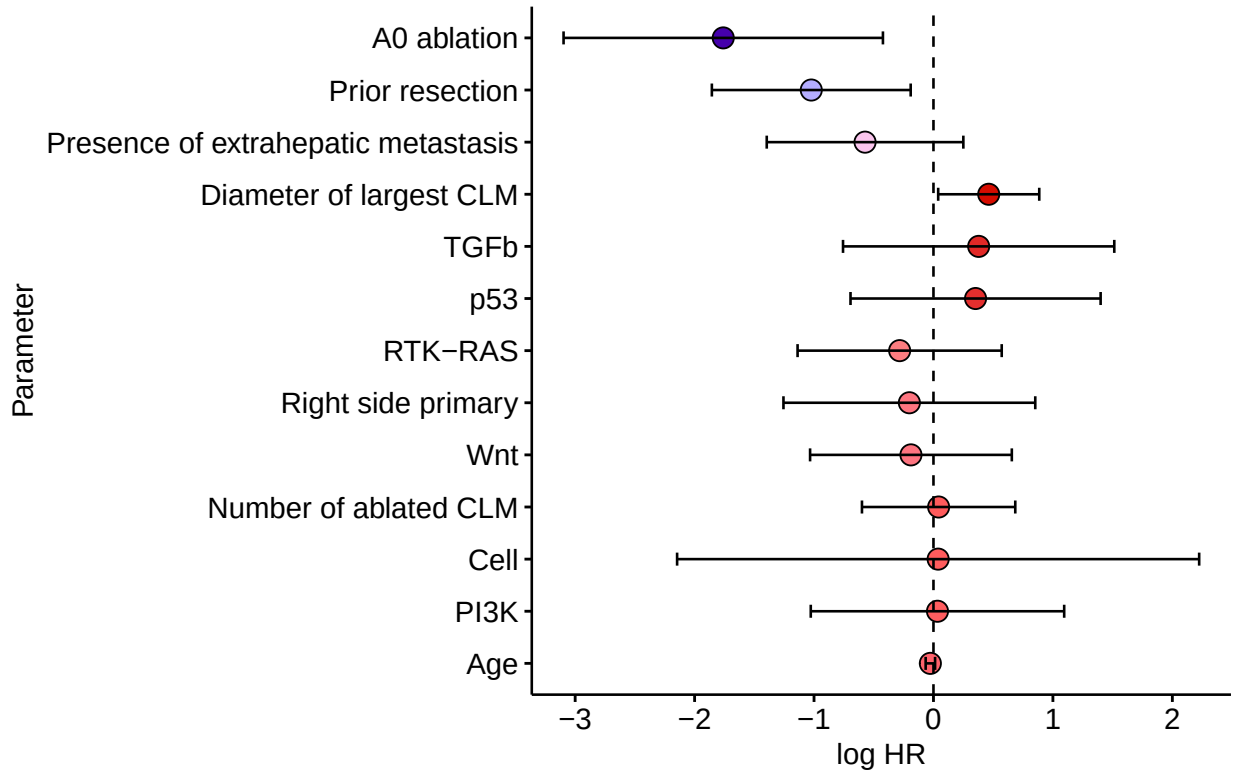

```
ltp.tbl <- ltp.multi %>% tbl_regression(
  label = list(age_at_procedure ~ "Age",
               prior_resection ~ "Prior resection",
               a0_ablation ~ "A0 ablation",
               max_diameter ~ "Diameter of largest CLM",
               nr_of_tumors ~ "Number of ablated CLM",
               right_side ~ "Right side primary",
               ehm ~ "Presence of extrahepatic metastasis",
               p53 ~ "p53",
               ras ~ "RTK-RAS",
               wnt ~ "Wnt",
               tgfb ~ "TGFb",
               pi3k ~ "PI3K",
               cell ~ "Cell"),
```

```
  exponentiate = TRUE,
  show_single_row = all_categorical(),
  pvalue_fun = function(x) style_pvalue(x, digits = 2),
  estimate_fun = function(x) style_ratio(x, digits = 2)
)

ltp.tbl
```

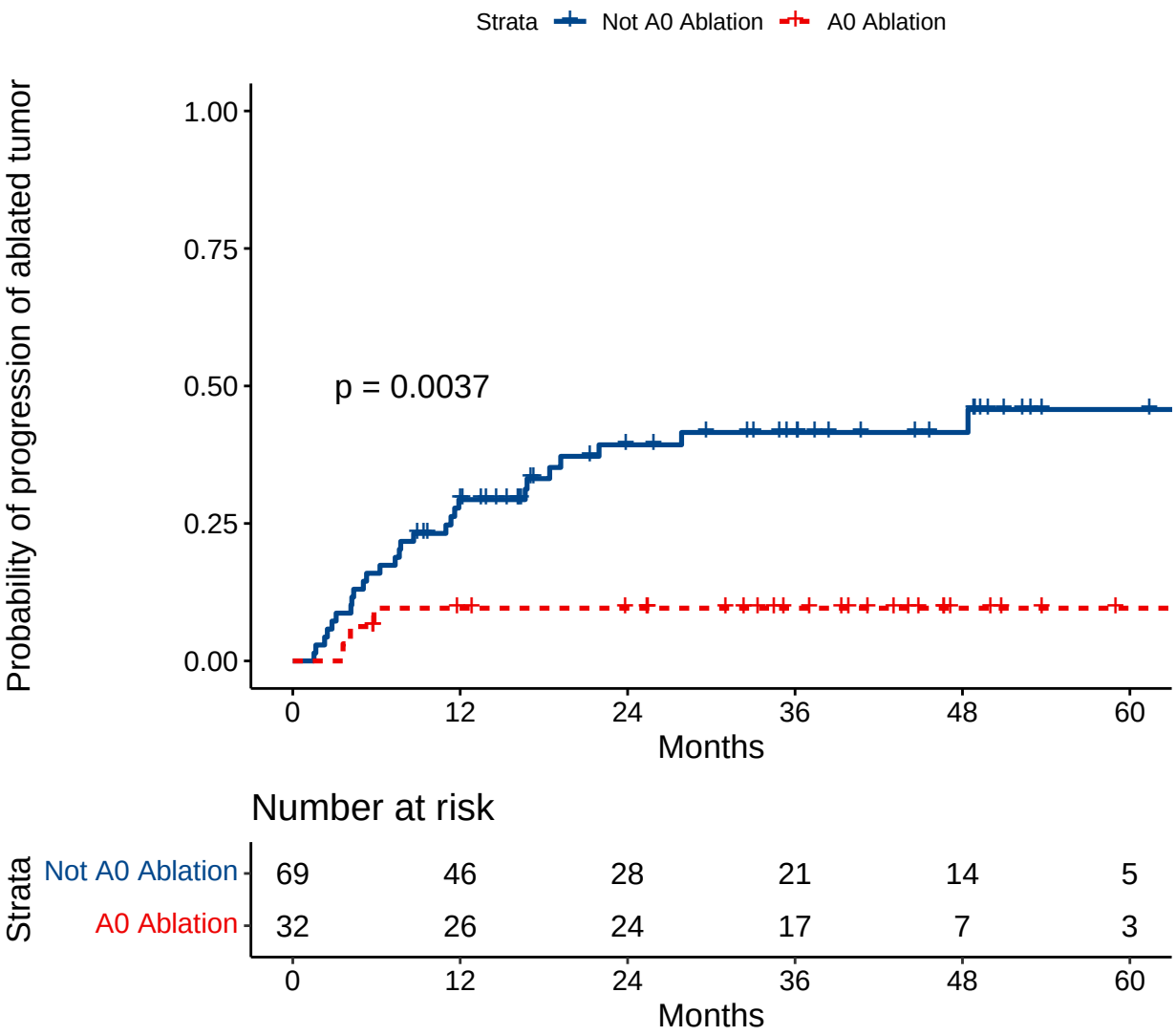
Characteristic	HR	95% CI	p-value
Age	0.97	0.94, 1.01	0.20
Prior resection	0.36	0.16, 0.83	0.016
Right side primary	0.82	0.28, 2.34	0.71
Diameter of largest CLM	1.59	1.04, 2.42	0.032
Number of ablated CLM	1.04	0.55, 1.98	0.90
Presence of extrahepatic metastasis	0.56	0.25, 1.28	0.17
A0 ablation	0.17	0.05, 0.66	0.010
p53	1.42	0.50, 4.05	0.51
RTK-RAS	0.75	0.32, 1.77	0.52
Wnt	0.83	0.36, 1.93	0.66
TGFb	1.46	0.47, 4.54	0.51
PI3K	1.03	0.36, 2.99	0.95
Cell	1.04	0.12, 9.25	0.97

```
ltp.tbl %>% as_gt() %>% gtsave("out/ltp.rtf")
```

```
ltp.tbl$table_body %>%
  select(var_label, estimate, conf.low, conf.high) %>%
  mutate(estimate = log(estimate),
         conf.low = log(conf.low),
         conf.high = log(conf.high)) %>%
  arrange(abs(estimate)) %>%
  ggdotplot(., x = 'var_label', y = 'estimate',
            fill = "estimate",
            orientation = 'horizontal',
            xlab = "Parameter", ylab = "log HR") +
  scale_fill_gsea() +
  guides(fill="none") +
  geom_errorbar(aes(group = var_label, ymax = conf.high, ymin = conf.low),
                position = position_dodge(width = 0.8), width = 0.25) +
  geom_hline(yintercept = 0, linetype = "dashed")
```



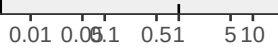

```
ggsurvplot(survfit(Surv(ltpfs, ltp) ~ a0_ablation,
                   data = data[data$mutation.analysis,]),
          pval = TRUE, risk.table = TRUE, conf.int = FALSE,
          pval.coord = c(3, 0.5),
          data = data[data$mutation.analysis,],
          legend.labs = c("Not A0 Ablation", "A0 Ablation"),
          xlim = c(0, 60), ylim = c(0, 1), break.time.by = 12, xlab = "Months",
          ylab = "Probability of progression of ablated tumor",
          palette = "lancet", fun = "event",
          linetype = c("strata"))
```


Strata
Not A0 Ablation
A0 Ablation
Probability of progression of ablated tumor
1.00
0.75
0.50
0.25
0.00
p = 0.0037
0
12
24
36
48
60
Months
Number at risk
Strata
Not A0 Ablation
A0 Ablation
69
46
28
21
14
5
32
26
24
17
7
3
0
12
24
36
48
60
Months

6 Salvage LRT

```
m.repeat.lrt <- glm(repeat_lrt ~ age_at_procedure + prior_resection +
                            ehm + right_side +
                            p53 + ras + wnt + tgfb + pi3k + notch + cell,
           data = data.ihp,
           family = 'binomial')

forest_model(model = m.repeat.lrt,
             exponentiate = TRUE,
             format_options = model.format)
```

Variable	N	Odds ratio		p
age_at_procedure	75		1.03 (0.98, 1.09)	0.256
prior_resection	75		2.71 (0.86, 9.35)	0.097
ehm	75		0.67 (0.21, 2.10)	0.496
right_side	75		1.02 (0.26, 4.13)	0.975
p53	75		0.48 (0.12, 1.71)	0.274
ras	75		0.38 (0.11, 1.20)	0.106
wnt	75		5.80 (1.94, 19.52)	0.003
tgfb	75		0.62 (0.13, 2.83)	0.531
pi3k	75		1.54 (0.31, 8.90)	0.605
notch	75		0.17 (0.01, 2.18)	0.186
cell	75		0.49 (0.04, 5.86)	0.554



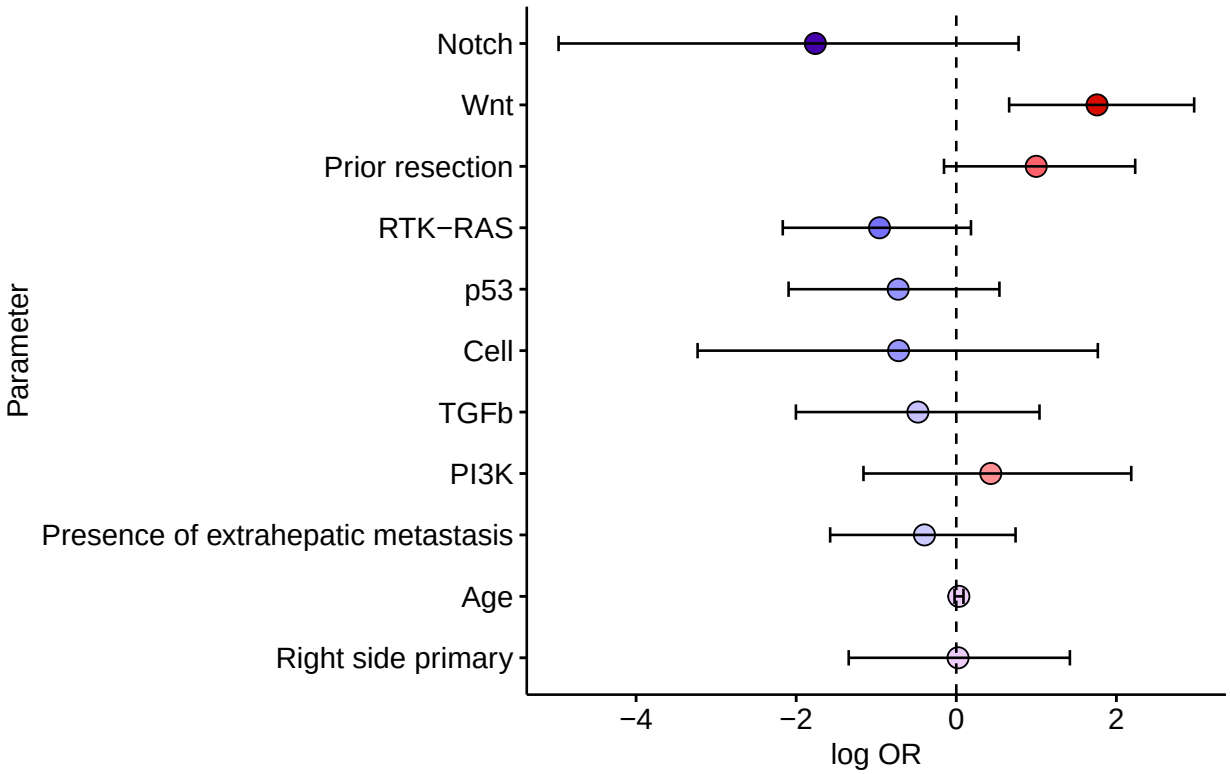

```
repeat.lrt.tbl <- m.repeat.lrt %>% tbl_regression(
  label = list(age_at_procedure ~ "Age",
               prior_resection ~ "Prior resection",
               right_side ~ "Right side primary",
               ehm ~ "Presence of extrahepatic metastasis",
               p53 ~ "p53",
               ras ~ "RTK-RAS",
               wnt ~ "Wnt",
               tgfb ~ "TGFb",
               pi3k ~ "PI3K",
               notch ~ "Notch",
               cell ~ "Cell"),
  exponentiate = TRUE,
  show_single_row = all_categorical(),
```

```
  pvalue_fun = function(x) style_pvalue(x, digits = 2),
  estimate_fun = function(x) style_ratio(x, digits = 2)
)

repeat.lrt.tbl
```

Characteristic	OR	95% CI	p-value
Age	1.03	0.98, 1.09	0.26
Prior resection	2.71	0.86, 9.35	0.10
Presence of extrahepatic metastasis	0.67	0.21, 2.10	0.50
Right side primary	1.02	0.26, 4.13	0.97
p53	0.48	0.12, 1.71	0.27
RTK-RAS	0.38	0.11, 1.20	0.11
Wnt	5.80	1.94, 19.5	0.003
TGFb	0.62	0.13, 2.83	0.53
PI3K	1.54	0.31, 8.90	0.61
Notch	0.17	0.01, 2.18	0.19
Cell	0.49	0.04, 5.86	0.55

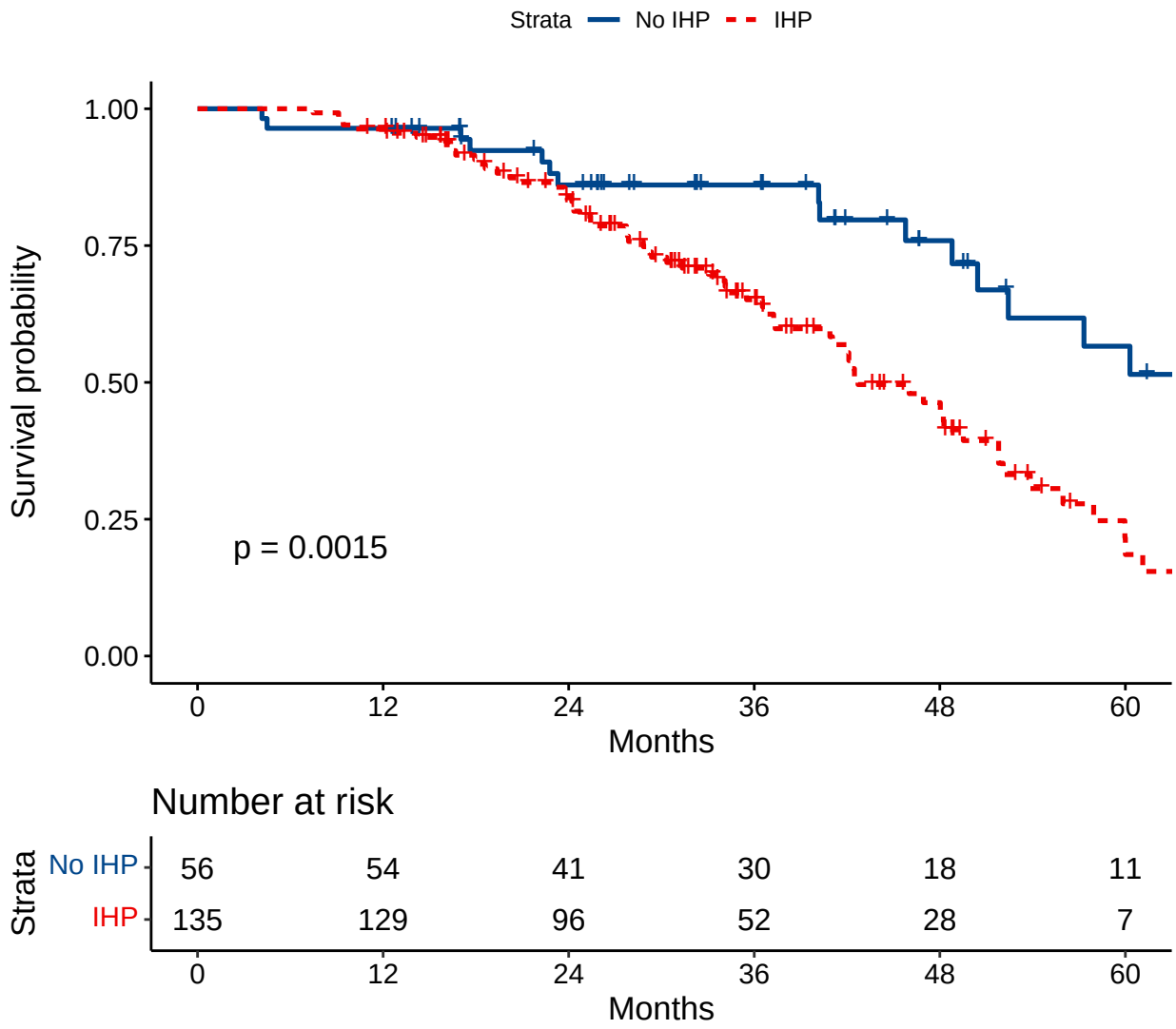
```
repeat.lrt.tbl %>% as_gt() %>% gtsave("out/repeat.lrt.rtf")
```

```
repeat.lrt.tbl$table_body %>%
  select(var_label, estimate, conf.low, conf.high) %>%
  mutate(estimate = log(estimate),
         conf.low = log(conf.low),
         conf.high = log(conf.high)) %>%
  arrange(abs(estimate)) %>%
  ggdotplot(., x = 'var_label', y = 'estimate',
            fill = "estimate",
            orientation = 'horizontal',
            xlab = "Parameter", ylab = "log OR") +
  scale_fill_gsea() +
  guides(fill="none") +
  geom_errorbar(aes(group = var_label, ymax = conf.high, ymin = conf.low),
                position = position_dodge(width = 0.8), width = 0.25) +
  geom_hline(yintercept = 0, linetype = "dashed")
```


Notch
Wnt
Prior resection
RTK−RAS
p53
Cell
TGFb
PI3K
Presence of extrahepatic metastasis
Age
Right side primary
Parameter
−4
−2
0
2
log OR

6.1 Survival following IHP

```
os.plot <- ggsurvplot(survfit(Surv(os_m, deceased) ~ ihp_any, data = data),
          pval = TRUE, risk.table = TRUE, conf.int = FALSE,
          data = data,
          legend.labs = c("No IHP", "IHP"),
          xlim = c(0, 60), break.time.by = 12, xlab = "Months",
          palette = "lancet",
          linetype = c("strata"))+
    guides(color = guide_legend(override.aes = list(shape = NA)))

os.plot
```

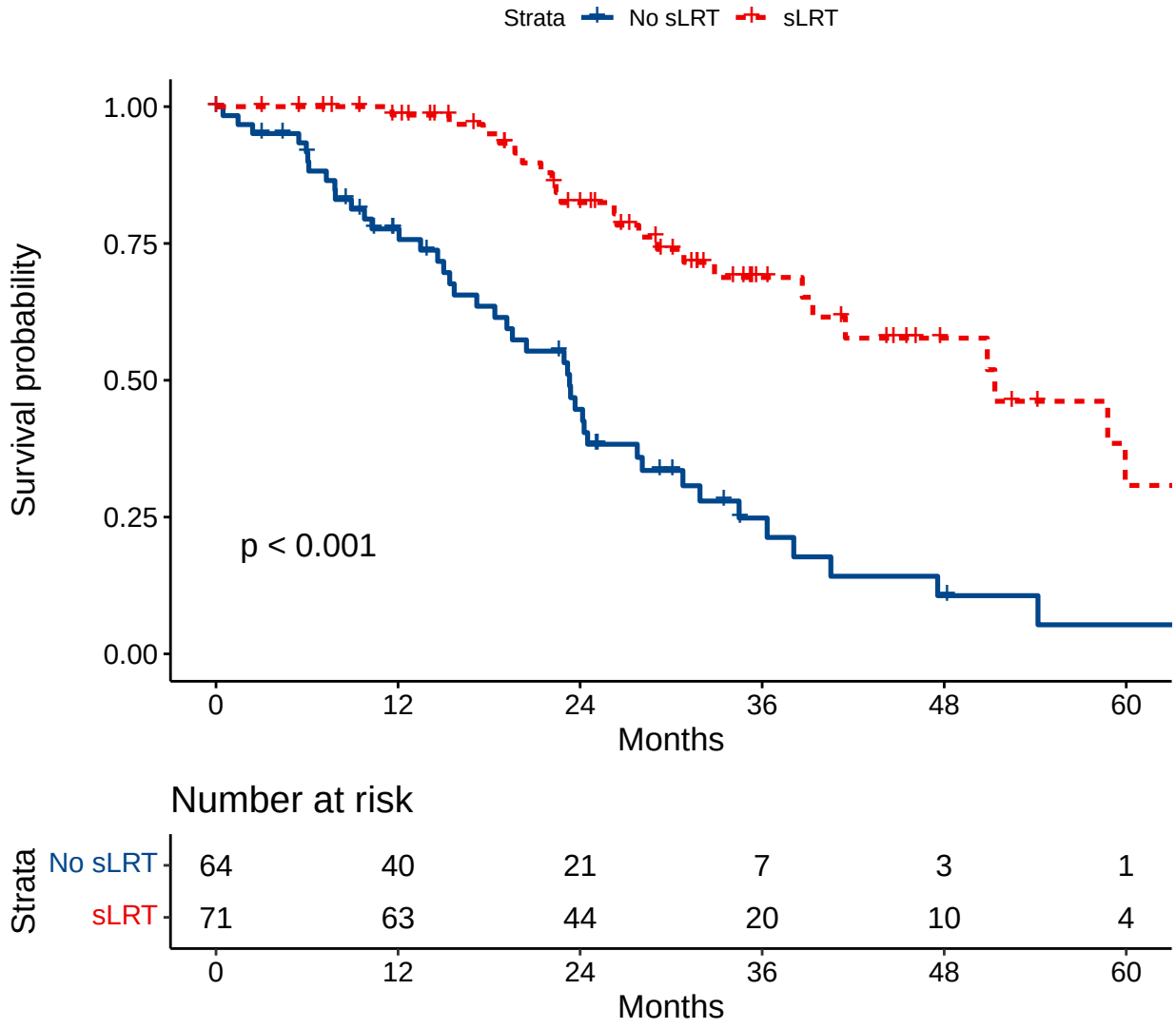



```
os_m.surv <- survfit(Surv(os_m, deceased) ~ ihp_any, data = data)
print(os_m.surv)
```

```
## Call: survfit(formula = Surv(os_m, deceased) ~ ihp_any, data = data)
##
##                 n events median 0.95LCL 0.95UCL
## ihp_any=FALSE  56     17   70.3    52.4      NA
## ihp_any=TRUE  135     65   42.7    40.9    51.8
```

6.2 Survival after IHP - salvage LRT vs. no salvage LRT

```
os2.plot <- ggsurvplot(survfit(Surv(os_ihp_m, deceased) ~ repeat_lrt, data = data.ihp),
         pval = "p < 0.001", risk.table = TRUE, conf.int = FALSE,
         data = data.ihp,
         legend.labs = c("No sLRT", "sLRT"),
         xlim = c(0, 60), break.time.by = 12, xlab = "Months",
         palette = "lancet",
         linetype = c("strata"))

os2.plot
```

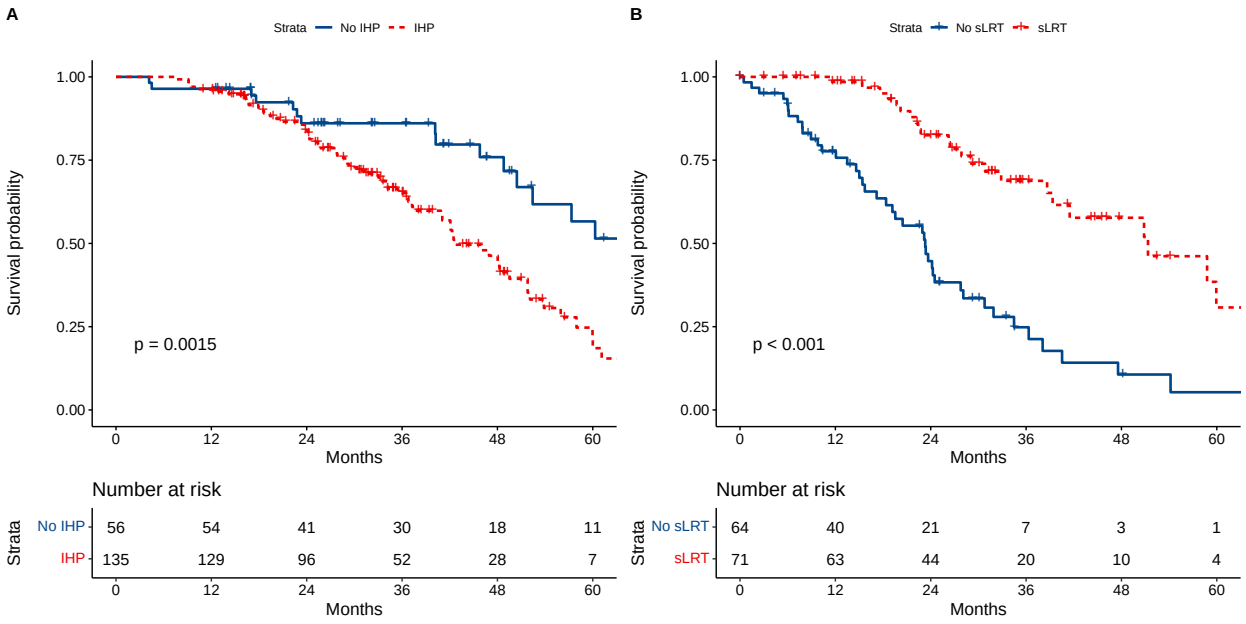



```
os2_m.surv <- survfit(Surv(os_ihp_m, deceased) ~ repeat_lrt, data = data.ihp)
print(os2_m.surv)
```

```
## Call: survfit(formula = Surv(os_ihp_m, deceased) ~ repeat_lrt, data = data.ihp)
##
##                   n events median 0.95LCL 0.95UCL
## repeat_lrt=FALSE 64     42   23.3    18.4    28.1
## repeat_lrt=TRUE  71     23   51.3    39.3      NA
```

6.2.1 Combined plot

```
ggarrange(os.plot$plot, os2.plot$plot, os.plot$table, os2.plot$table,
          labels = c("A", "B"),
          ncol = 2, nrow = 2, heights = c(2.5, 0.8),
          align = "v")
```



7 Frequency of mutations

```
data.grouped <- data %>% filter(mutation.analysis) %>%
  group_by(race) %>%
    summarise(
        n = n(),
        n_tot = n(),
        p53 = sum(p53),
        Wnt = sum(wnt),
        "RTK-RAS" = sum(ras),
        PI3K = sum(pi3k),
        TGFb = sum(tgfb),
        Notch = sum(notch),
        "Cell Cycle" = sum(cell),
    )
data.grouped
```

race	n	n_tot	p53	Wnt	RTK-RAS	PI3K	TGFb	Notch	Cell Cycle
af	10	10	8	6	6	4	1	0	0
as	5	5	4	2	2	1	0	0	0
cauc	77	77	57	51	35	10	13	4	3
his	6	6	6	3	2	1	1	1	1
NA	3	3	0	2	3	1	1	0	0

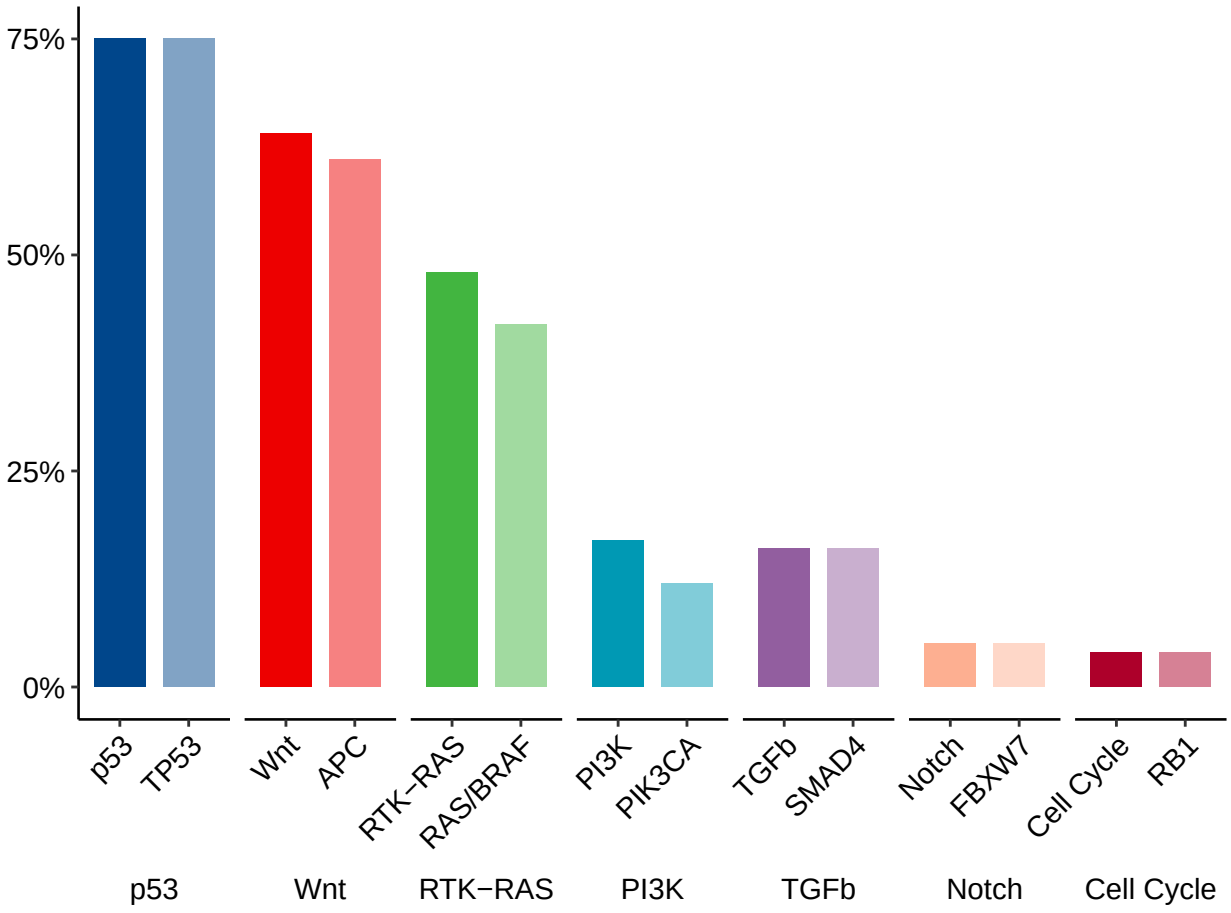
```
data.grouped.wide <- gather(data.grouped, key = type_of_mutation, nr_mutated,
       p53,Wnt,"RTK-RAS",PI3K,TGFb,Notch,"Cell Cycle",
       factor_key=TRUE, na.rm = TRUE)

data.grouped.wide <- within(data.grouped.wide, {
  nr_mutated_perc <- (nr_mutated / n) * 100.0

  race <- ifelse(race == "his", "Hi", race)
  race <- ifelse(race == "cauc", "Ca", race)
  race <- ifelse(race == "as", "As", race)
  race <- ifelse(race == "af", "Af", race)
  race <- factor(race, levels = c("Ca", "Hi", "Af", "As"))
})
```

7.1 Number of mutations per pathway with predominant gene

```
data.mut <- data %>% filter(mutation.analysis)

data.plot <- data.frame(pathway = c("p53", "p53", "Wnt", "Wnt", "RTK-RAS", "RTK-RAS",
                                    "PI3K", "PI3K", "TGFb", "TGFb", "Notch", "Notch",
                                    "Cell Cycle", "Cell Cycle"),
                        mutation = c("p53", "TP53", "Wnt", "APC", "RTK-RAS", "RAS/BRAF",
                                     "PI3K", "PIK3CA", "TGFb", "SMAD4", "Notch", "FBXW7",
                                     "Cell Cycle", "RB1"),
```

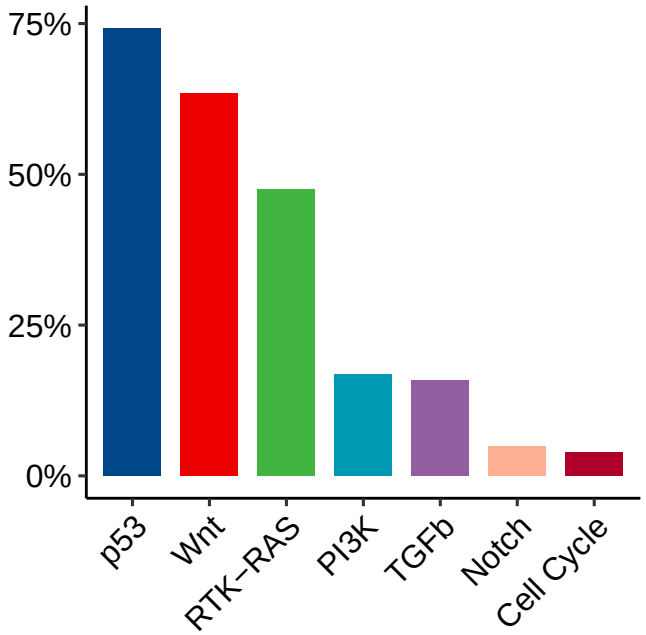
```
                        nr_mutated = c(sum(data.mut$p53), sum(data.mut$tp53),
                                       sum(data.mut$wnt), sum(data.mut$apc),
                                       sum(data.mut$ras), sum(data.mut$rasbraf),
                                       sum(data.mut$pi3k), sum(data.mut$pik3ca),
                                       sum(data.mut$tgfb), sum(data.mut$smad4),
                                       sum(data.mut$notch), sum(data.mut$fbxw7),
                                       sum(data.mut$cell), sum(data.mut$rb1))) %>%
  mutate(nr_mutated_perc = nr_mutated / sum(data.mut$mutation.analysis))

data.plot$mutation <- factor(data.plot$mutation,
                             levels = c("p53", "TP53", "Wnt", "APC", "RTK-RAS", "RAS/BRAF",
                                        "PI3K", "PIK3CA", "TGFb", "SMAD4", "Notch", "FBXW7",
                                        "Cell Cycle", "RB1"))

data.plot$pathway <- factor(data.plot$pathway,
                            levels = c("p53", "Wnt", "RTK-RAS",
                                       "PI3K", "TGFb", "Notch", "Cell Cycle"))
```

```
ggplot(data = data.plot, aes(x = mutation, y = nr_mutated,
                                     fill = mutation)) +
    geom_bar(stat="identity", width = 0.75, position = position_dodge(width=1)) +
    facet_grid(~pathway, scales = "free_x", space = "free_x", switch = "x") +
    scale_fill_manual(values = rbind(pal_lancet()(7), pal_lancet(alpha = 0.5)(7))) +
    scale_y_continuous(breaks = seq(0, 100, 25), labels = paste(seq(0, 100, 25), "%", sep="")) +
    theme_pubr() +
    theme(strip.placement = "outside",
          axis.text.x = element_text(size = 12, angle = 45, hjust = 1),
        axis.title = element_blank()) +
    theme(strip.text.x = element_text( size = 12, color = "black"),
          strip.background = element_blank()) +
    theme(legend.position = "None")
```


75%
50%
25%
0%
p53
TP53
Wnt
APC
RTK−RAS
RAS/BRAF
PI3K
PIK3CA
TGFb
SMAD4
Notch
FBXW7
Cell Cycle
RB1
p53
Wnt
RTK−RAS
PI3K
TGFb
Notch
Cell Cycle

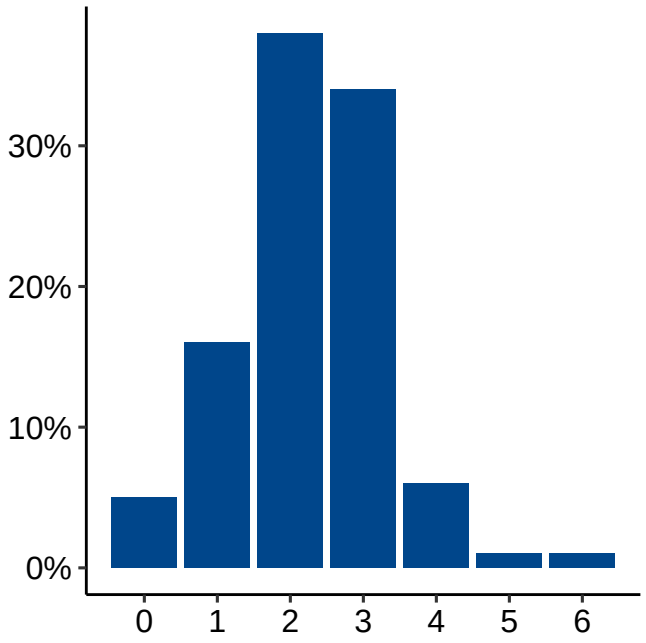
7.2 Number of mutations per pathway

```
data.plot <- data.grouped.wide %>%
  group_by(type_of_mutation) %>%
  summarise(
    n = n(),
    n_tot = sum(n_tot),
    nr_mutated = sum(nr_mutated)
  )
data.plot$nr_mutated_perc <- (data.plot$nr_mutated / data.plot$n_tot) * 100
ggplot(data = data.plot, aes(x = type_of_mutation, y = nr_mutated_perc,
                                    fill = type_of_mutation)) +
    geom_bar(stat="identity", width = 0.75, position = position_dodge(width=1)) +
    scale_fill_lancet() +
    scale_y_continuous(breaks = seq(0, 100, 25), labels = paste(seq(0, 100, 25), "%", sep="")) +
    theme_pubr() +
    theme(strip.placement = "outside",
          axis.text.x = element_text(size = 12, angle = 45, hjust = 1),
        axis.title = element_blank()) +
    theme(strip.text.x = element_text( size = 12, color = "black"),
          strip.background = element_blank()) +
    theme(legend.position = "None")
```



7.3 Frequency of mutations

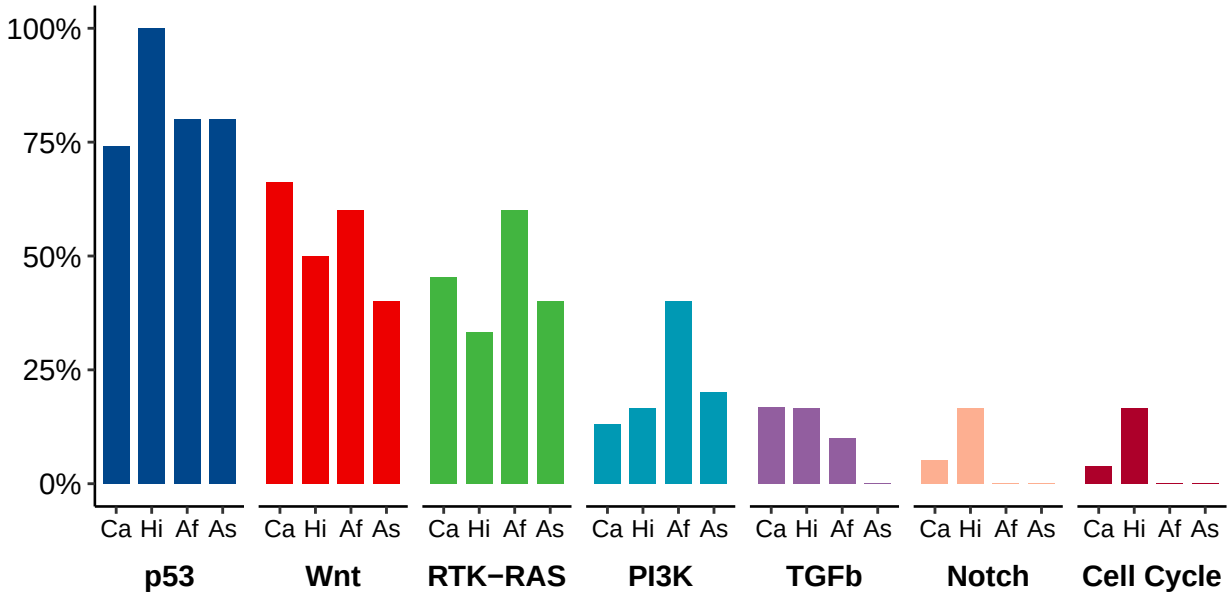
```
ggplot(data = data, aes(x = num_altered_paths, fill = "num_altered_paths")) +
    geom_histogram(stat="count") +
    scale_fill_lancet() +
    scale_y_continuous(breaks = seq(0, 50, 10), labels = paste(seq(0, 50, 10), "%", sep="")) +
    scale_x_continuous(breaks = seq(0, 6, 1)) +
    xlab("Nr of altered pathways") +
    theme_pubr() +
    theme(strip.placement = "outside",
          axis.text.x = element_text(size = 12),
        axis.title = element_blank()) +
    theme(strip.text.x = element_text( size = 12, color = "black"),
          strip.background = element_blank()) +
    theme(legend.position = "None")
```



7.4 Muation per race/ethnicity

```
data.grouped.wide <- data.grouped.wide %>% filter(!is.na(race))
data.grouped.wide$race <- as.factor(data.grouped.wide$race)

ggplot(data = data.grouped.wide, aes(x = race, y = nr_mutated_perc,
                                     fill = type_of_mutation)) +
    geom_bar(stat="identity", width = 0.75, position = position_dodge(width=1)) +
    facet_grid(~type_of_mutation, scales = "free_x", space = "free_x", switch = "x") +
    scale_fill_lancet() +
    scale_y_continuous(breaks = seq(0, 100, 25), labels = paste(seq(0, 100, 25), "%", sep="")) +
    theme_pubr() +
```

```
  theme(strip.placement = "outside",
        axis.text.x = element_text(size = 10),
      axis.title = element_blank()) +
  theme(strip.text.x = element_text( size = 12, color = "black", face = "bold"),
        strip.background = element_blank()) +
  theme(legend.position = "None")
```



8 Citation

```
citation('ggplot2')
```

```
##
## To cite ggplot2 in publications, please use:
##
##   H. Wickham. ggplot2: Elegant Graphics for Data Analysis.
##   Springer-Verlag New York, 2016.
##
## A BibTeX entry for LaTeX users is
##
##   @Book{,
##     author = {Hadley Wickham},
##     title = {ggplot2: Elegant Graphics for Data Analysis},
##     publisher = {Springer-Verlag New York},
##     year = {2016},
##     isbn = {978-3-319-24277-4},
##     url = {https://ggplot2.tidyverse.org},
##   }
```

```
citation('ggpubr')
```

```
##
## To cite package 'ggpubr' in publications use:
##
##   Alboukadel Kassambara (2020). ggpubr: 'ggplot2' Based Publication
##   Ready Plots. R package version 0.4.0.
##   https://CRAN.R-project.org/package=ggpubr
##
## A BibTeX entry for LaTeX users is
##
##   @Manual{,
##     title = {ggpubr: 'ggplot2' Based Publication Ready Plots},
##     author = {Alboukadel Kassambara},
##     year = {2020},
##     note = {R package version 0.4.0},
##     url = {https://CRAN.R-project.org/package=ggpubr},
##   }
```

```
citation('tidyverse')
```

```
##
##   Wickham et al., (2019). Welcome to the tidyverse. Journal of Open
##   Source Software, 4(43), 1686, https://doi.org/10.21105/joss.01686
##
## A BibTeX entry for LaTeX users is
##
##   @Article{,
##     title = {Welcome to the {tidyverse}},
##     author = {Hadley Wickham and Mara Averick and Jennifer Bryan and Winston Chang and Lucy D'Agostin
```

```
##     year = {2019},
##     journal = {Journal of Open Source Software},
##     volume = {4},
##     number = {43},
##     pages = {1686},
##     doi = {10.21105/joss.01686},
##   }
```

```
citation()
```

```
##
## To cite R in publications use:
##
##   R Core Team (2021). R: A language and environment for statistical
##   computing. R Foundation for Statistical Computing, Vienna, Austria.
##   URL https://www.R-project.org/.
##
## A BibTeX entry for LaTeX users is
##
##   @Manual{,
##     title = {R: A Language and Environment for Statistical Computing},
##     author = {{R Core Team}},
##     organization = {R Foundation for Statistical Computing},
##     address = {Vienna, Austria},
##     year = {2021},
##     url = {https://www.R-project.org/},
##   }
##
## We have invested a lot of time and effort in creating R, please cite it
## when using it for data analysis. See also 'citation("pkgname")' for
## citing R packages.
```