Survival analysis of patients with chondrosarcoma

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This script performs a survival analysis of patients with chondrosarcoma within the E-MTAB-7264 dataset of chondrosarcoma tumors from 102 patients.

The 1st part consists of analyze the impact of immune cells types expression on the survival probabilities to see the types that have a positive or negative impact. The 2nd part is about the impact of CTA expression and finally the last part is about survival analysis from clustering of CTA expression that impact the survival probabilities (script 5) to see the impact of CTA and correlate CTA and immune cells.

These 2 analysis are separated by select all patients or conventional patients because dedifferentied tumors are different and have an impact on the survival probabilities. The survival probabilities are comput thanks to Cox regression and log rank test.

Load librairies

```r
library(dplyr)
library(survival)
library(forestplot)
library(survminer)
library(ggplot2)
library(ggsurvfit)
library(gridExtra)
library(tidyr)
```

Functions

```r
# Function to apply coxph model per columns
apply_coxph_model <- function(df) {
    # Empty df
    results <- data.frame()

    # Loop on each columns to apply coxph
    for (col in colnames(df)[3:ncol(df)]) {
        model <- coxph(Surv(OS.delay, OS.event) ~ df[[col]],
            data = df)

        # Extract results
        exp_coef <- summary(model)$coefficients[, "exp(coef)"]
        lower_ci <- summary(model)$conf.int[, "lower .95"]
        upper_ci <- summary(model)$conf.int[, "upper .95"]
        p_value <- summary(model)$coefficients[, "Pr(>|z|)"]
        results <- rbind(results, data.frame(Variable = col,
            HR = exp_coef, LowerCI = lower_ci, UpperCI = upper_ci,
            Pvalue = p_value))
    }
    return(results)
}

# Function to create Kaplan-Meier plot
plot_km <- function(mod, df) {
    km_plot <- ggsurvplot(mod, data = df, pval = TRUE, conf.int = FALSE,
        ggtheme = theme_bw(), palette = c("#E7B800", "#2E9FDF",
            "#FF6F61", "#4EBB92"))
    return(km_plot$plot)
}

# Function to generate forest plot from coxph analysis from
# the function apply_coxph_model
generate_forestplot <- function(data, Type) {
    if (Type == TRUE) {
        # Order by Signature and p-value
        data <- data[order(data$Signature, data$Pvalue), ]
        data$Pvalue <- sprintf("%.4f", data$Pvalue)
        data %>%
            forestplot(labeltext = c(Signature, Variable, Pvalue),
                mean = HR, lower = LowerCI, upper = UpperCI,
                grid = TRUE, zero = 1, col = fpColors(box = "black",
                  line = "black"), hrzl_lines = TRUE, title = "Forest Plot for Cox Model",
                txt_gp = fpTxtGp(label = gpar(fontsize = 8)),
                ci.vertices = TRUE, boxsize = 0.1) %>%
            fp_set_zebra_style("#EFEFEF") %>%
            fp_add_header(Signature = c("", "Cell type"), Variable = c("",
                "Genes"), Pvalue = c("", "p-value"))
    } else {
        # Order by p-value
        data <- data[order(data$Pvalue), ]
        data$Pvalue <- sprintf("%.4f", data$Pvalue)
        data %>%
            forestplot(labeltext = c(Variable, Pvalue), mean = HR,
                lower = LowerCI, upper = UpperCI, grid = TRUE,
                zero = 1, col = fpColors(box = "black", line = "black"),
                hrzl_lines = TRUE, title = "Forest Plot for Cox Model",
                txt_gp = fpTxtGp(label = gpar(fontsize = 8)),
                ci.vertices = TRUE, boxsize = 0.1) %>%
            fp_set_zebra_style("#EFEFEF") %>%
            fp_add_header(Variable = c("", "Genes"), Pvalue = c("",
                "p-value"))
    }
}

# Function to calculate Z scores
calculate_z_scores <- function(df_input, col) {
    # Exclude col PROBEID SYMBOL and CTA
    data_values <- df_input[, -c(col)]

    # Calculate Z-scores
    z_scores_row <- t(scale(t(data_values)))

    # Add columns
    df_z_scores <- cbind(df_input[, c(col)], z_scores_row)

    # Return df
    return(df_z_scores)
```

```
}
```

Load and format data

```
# Read the metadata and select individuals that have
# survival data
df_metadata <- read.table("../results/metadata.tsv", sep = "\t",
    header = TRUE, check.names = FALSE, dec = ",")
df_metadata_surv <- df_metadata[, c("Patient", "OS.delay", "OS.event")]
df_metadata_surv <- na.omit(df_metadata_surv)

# Conventional chondrosarcoma
df_metadata_conv <- df_metadata[df_metadata$Histology != "N/A" &
    df_metadata$Histology != "benign" & df_metadata$Histology !=
    "dedifferentiated", ]
df_metadata_surv_conv <- df_metadata_conv[, c("Patient", "OS.delay",
    "OS.event")]
df_metadata_surv_conv <- na.omit(df_metadata_surv_conv)

# Read the data and select lines of the immune cells
# expression
df_imm_z_scores_82 <- read.table("../results/imm_sign_z_scores_82.tsv",
    sep = "\t", header = TRUE, row.names = 1)
df_imm_z_scores_82 <- as.data.frame(t(df_imm_z_scores_82))

# Read matrix
df_CTA_immune_whole_clean_avg_102 <- read.table("../results/whole_gene_int_CTA_sign_imm_clean.tsv",
    sep = "\t", header = TRUE, check.names = FALSE)
rownames(df_CTA_immune_whole_clean_avg_102) <- df_CTA_immune_whole_clean_avg_102$SYMBOL
df_CTA_immune_whole_clean_avg_82 <- df_CTA_immune_whole_clean_avg_102[,
    c("Signature", "CTA", colnames(df_CTA_immune_whole_clean_avg_102)[colnames(df_CTA_immune_whole_clea
        df_metadata_surv$Patient])]
df_z_scores_82 <- calculate_z_scores(df_CTA_immune_whole_clean_avg_82,
    c(1, 2))

# Matrix for conventional patients with survival metadata
df_CTA_immune_whole_clean_avg_63 <- df_CTA_immune_whole_clean_avg_102[,
    c("Signature", "CTA", colnames(df_CTA_immune_whole_clean_avg_102)[colnames(df_CTA_immune_whole_clea
        df_metadata_surv_conv$Patient])]
df_z_scores_63 <- calculate_z_scores(df_CTA_immune_whole_clean_avg_63,
    c(1, 2))

# Immune signature z scores for conv patients
df_imm_z_scores_63 <- read.table("../results/imm_sign_z_scores_63.tsv",
    sep = "\t", header = T)
df_imm_z_scores_63 <- as.data.frame(t(df_imm_z_scores_63))
```

I. Immune cells survival analysis

This part concern the survival analysis with the expression of the immune cells. This is to observe the impact of each cell types on the survival probabilities of the patients.

1) All chondrosarcoma types

a- Categorical data

Categorical data refer to HIGH or LOW expression based on the median of z-scores. If the score is > than median, HIGH is affected contrary to < than median, LOW is affected. In this section, we use Cox regression.

```
# Transform to categorical data and take continuous data
# Computing medians to have categorical data
medians_sign <- apply(df_imm_z_scores_82, 2, median)

# Add categorical data, LOW is for under expressed genes
# (under the median) and HIGH upreg
df_cat_all <- df_imm_z_scores_82
for (col in colnames(df_cat_all)) {
    new_column_name <- paste(col, "cat", sep = " ")
    df_cat_all[[new_column_name]] <- ifelse(df_imm_z_scores_82[[col]] >
        medians_sign[col], "HIGH", "LOW")
}

# Select categories
df_cat_all <- df_cat_all[, grep("cat", colnames(df_cat_all))]

# Replace space by _
colnames(df_cat_all) <- gsub(" ", "_", colnames(df_cat_all))

# Add col for merging
df_cat_all$Patient <- rownames(df_cat_all)

# Merge df
df_survival_all <- merge(df_metadata_surv, df_cat_all, by = "Patient")
rownames(df_survival_all) <- df_survival_all$Patient
df_survival_all <- df_survival_all[, -1]

# Continuous data
df_cont_all <- df_imm_z_scores_82

# Replace space by _
colnames(df_cont_all) <- gsub(" ", "_", colnames(df_cont_all))

# Merge df
df_cont_all$Patient <- rownames(df_cont_all)
df_survival_cont <- merge(df_metadata_surv, df_cont_all, by = "Patient")
rownames(df_survival_cont) <- df_survival_cont$Patient
df_survival_cont <- df_survival_cont[, -1]

# Apply coxph model on the categorical variables for the
# immune signatures
```

```r
results_cat <- apply_coxph_model(df_survival_all)
# write.table(results_cat, '../results/results_coxhp.tsv',
# sep = '\t', row.names = FALSE, quote = FALSE)
generate_forestplot(data = results_cat, Type = FALSE)
```

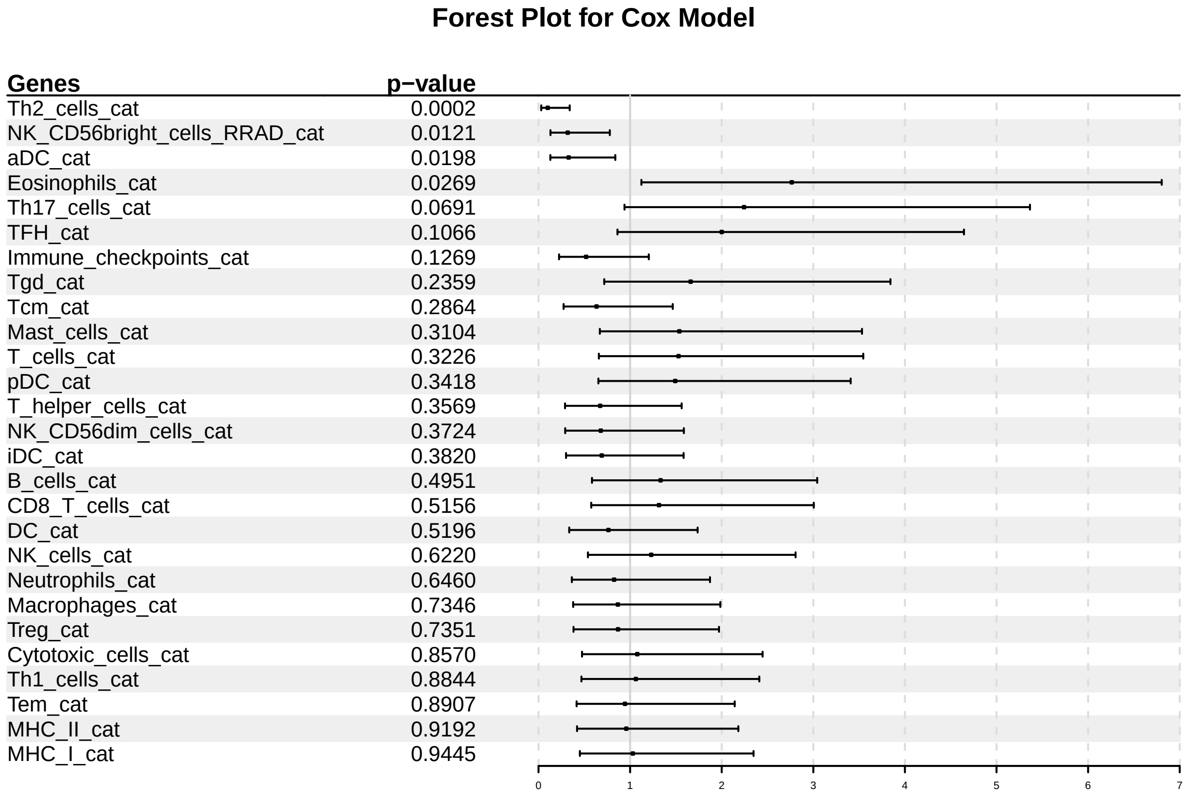


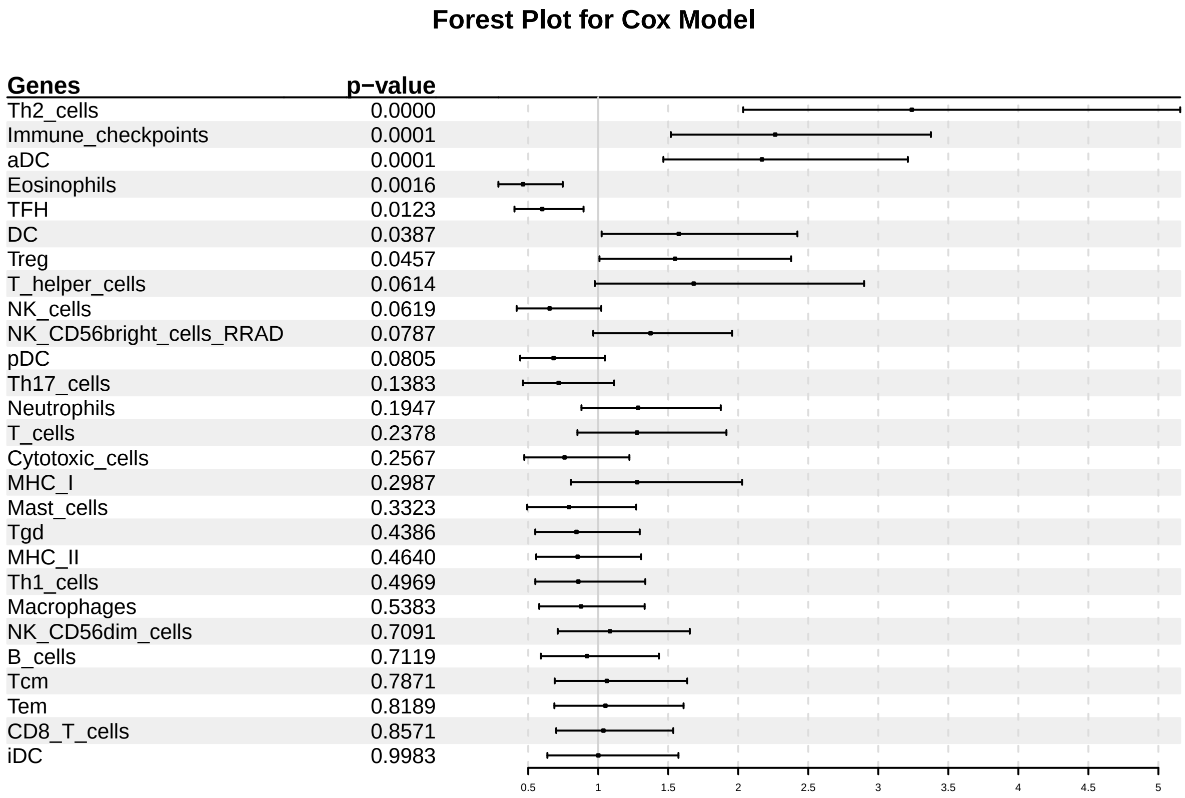
Figure 1: Forest plot for categorical immune cell types expression (n = 82)

We see that the p-value are high, maybe because of categorical data because Cox model rank the values.

b- Continuous data

In this section, we use z-scores data with Cox model.

```
# Apply coxph on continuous
results_cont <- apply_coxph_model(df_survival_cont)
# write.table(results_cont,
# '../results/results_coxph_var_cont.tsv', sep = '\t',
# row.names = FALSE, quote = FALSE)
# pdf('../results/figures/forest_plots/all_indiv/forest_plot_all_patients_var_cont.pdf')
generate_forestplot(results_cont, Type = FALSE)
```



Forest Plot for Cox Model

Genes	p−value
Th2_cells	0.0000
Immune_checkpoints	0.0001
aDC	0.0001
Eosinophils	0.0016
TFH	0.0123
DC	0.0387
Treg	0.0457
T_helper_cells	0.0614
NK_cells	0.0619
NK_CD56bright_cells_RRAD	0.0787
pDC	0.0805
Th17_cells	0.1383
Neutrophils	0.1947
T_cells	0.2378
Cytotoxic_cells	0.2567
MHC_I	0.2987
Mast_cells	0.3323
Tgd	0.4386
MHC_II	0.4640
Th1_cells	0.4969
Macrophages	0.5383
NK_CD56dim_cells	0.7091
B_cells	0.7119
Tcm	0.7871
Tem	0.8189
CD8_T_cells	0.8571
iDC	0.9983

Figure 2: Forest plot for continuous immune cell types expression (n = 82)

```
# dev.off()
```

The p-values are smaller than the model with categorical data so it's seems to be better with continuous data.

For all patients, we see that the hazard ratio (survival probabilities) are increased for Th2 cells, Immune checkpoints, aDC and Treg. So, more these cell types are present in the tumors, more the probability of death is higher, these cell types have a bad impact on survival. Contrary to eosinophils and DC, the HR decreased so more these cells are present in the tumors, the death probability decreased so they have a good impact on prognostic.

c- Kaplan-Meier plots

This part compute Kaplan-Meier plot based on log rank test that use categorical data to see the trend of survival for the 2 groups (HIGH or LOW expression of the cell types) This shows the graph for each significant HR ratio previously computed with Cox model.

```r
# Create Kaplan-Meier plots with categorical data
p1 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ DC_cat, data = df_survival_all),
    df_survival_all)
p2 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Eosinophils_cat,
    data = df_survival_all), df_survival_all)
p3 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Immune_checkpoints_cat,
    data = df_survival_all), df_survival_all)
p4 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ TFH_cat, data = df_survival_all),
    df_survival_all)
p5 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Th2_cells_cat,
    data = df_survival_all), df_survival_all)
p6 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Treg_cat, data = df_survival_all),
    df_survival_all)
p7 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ aDC_cat, data = df_survival_all),
    df_survival_all)
# pdf('../results/figures/other_plots/KM_plots_signif_cox_all_indiv.pdf',
# height = 20, width = 15)
grid.arrange(p1, p2, p3, p4, p5, p6, p7, ncol = 2)
```

```r
# dev.off()
```

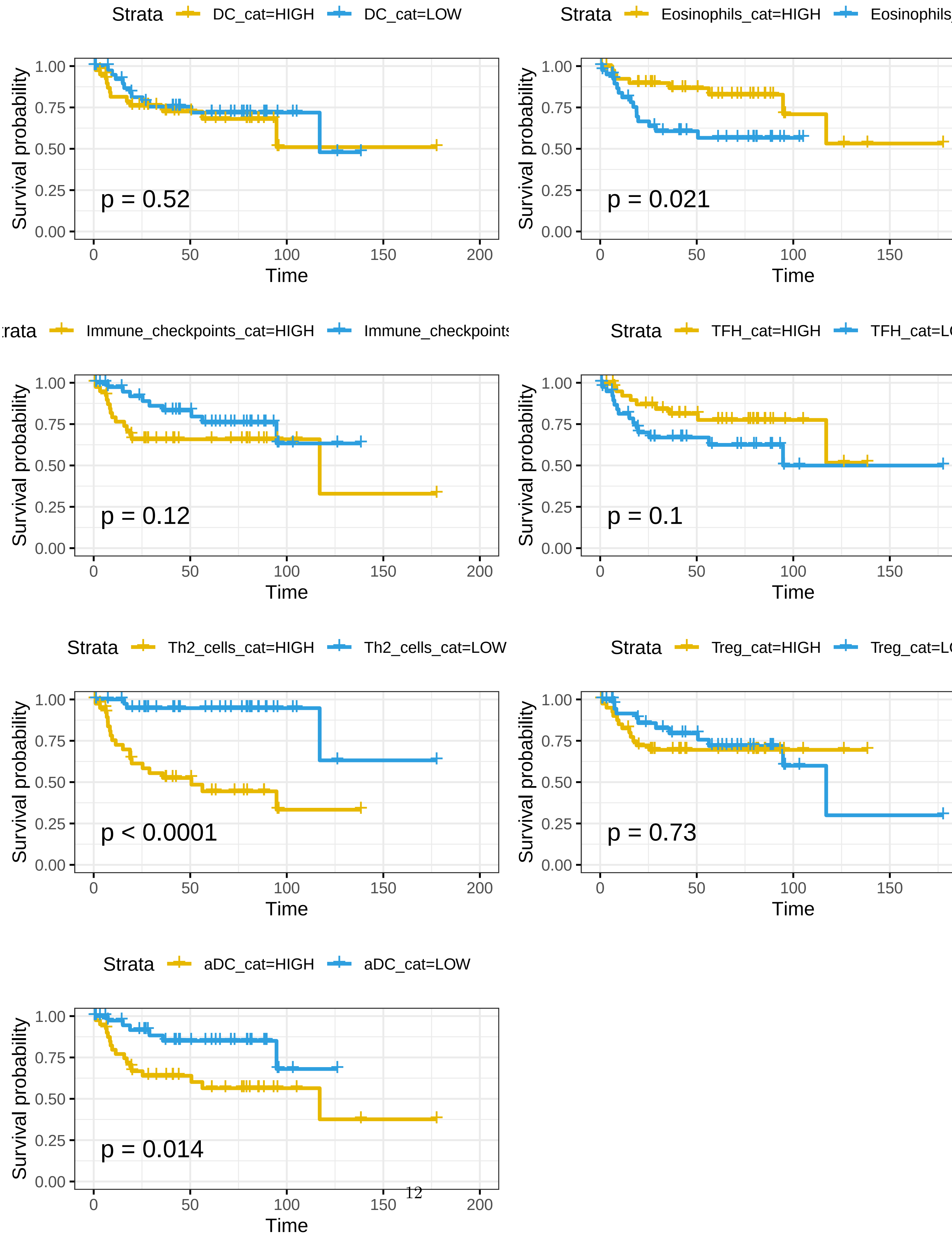


Figure 3: Kaplan-Meier plots for immune cell types (n = 82)

With log rank test, we see that only Th2 and eosinophils are significant.

d- Survival analysis for Th2 cells

In this part, we want to see the HR of the genes expressed by Th2 cells To determine if some genes have a dominant effect over others.

```
# List of genes expressed in th2 cells
l_genes_th2 <- c("ADCY1", "AHI1", "ANK1", "BIRC5", "CDC25C",
    "CDC7", "CENPF", "CXCR6", "DHFR", "EVI5", "GATA3", "GSTA4",
    "HELLS", "IL26", "LAIR2", "LIMA1", "MB", "MICAL2", "NEIL3",
    "PHEX", "PMCH", "PTGIS", "SLC39A14", "SMAD2", "SNRPD1", "WDHD1")

# Select rows in the expression matrix
df_expr_th2 <- df_z_scores_82[rownames(df_z_scores_82) %in% l_genes_th2,
    ]
df_expr_th2 <- df_expr_th2[, -c(1:2)]

# Apply coxph model
df_expr_th2 <- as.data.frame(t(df_expr_th2))
df_expr_th2 <- df_expr_th2[df_metadata_surv$Patient, ]
df_expr_th2$Patient <- rownames(df_expr_th2)
df_survival_th2 <- merge(df_metadata_surv, df_expr_th2, by = "Patient")
rownames(df_survival_th2) <- df_survival_th2$Patient
df_survival_th2 <- df_survival_th2[, -1]
results_th2 <- apply_coxph_model(df_survival_th2)

# Generate forest plot
# pdf('../results/figures/forest_plots/all_indiv/forest_plot_all_patients_th2.pdf')
generate_forestplot(results_th2, Type = FALSE)
```

```
# dev.off()
```

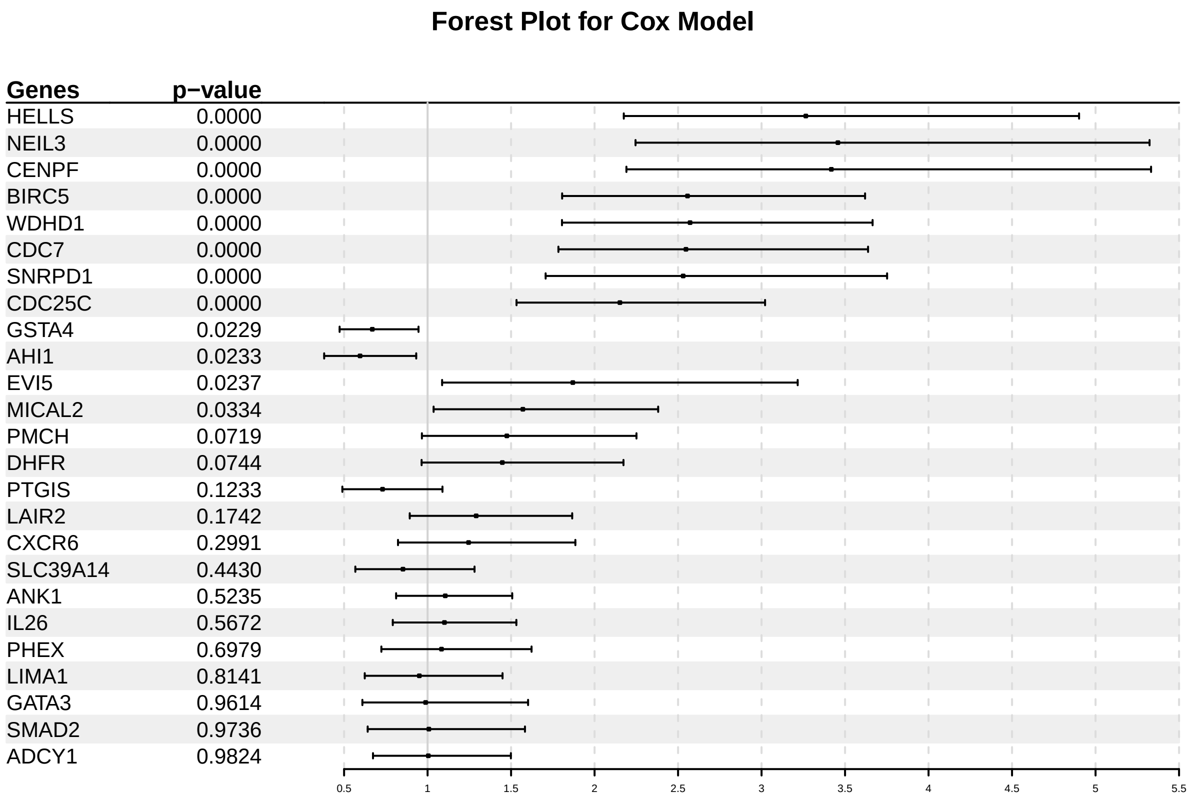


Figure 4: Forest plot for Th2 cells (n = 82)

So, we see that for th 12 significant genes, 10 have a ratio > 2 et only 2 genes have a small ratio. We now can confirm than the prese,ce of Th2 cells have a bad impact on the prognosis.

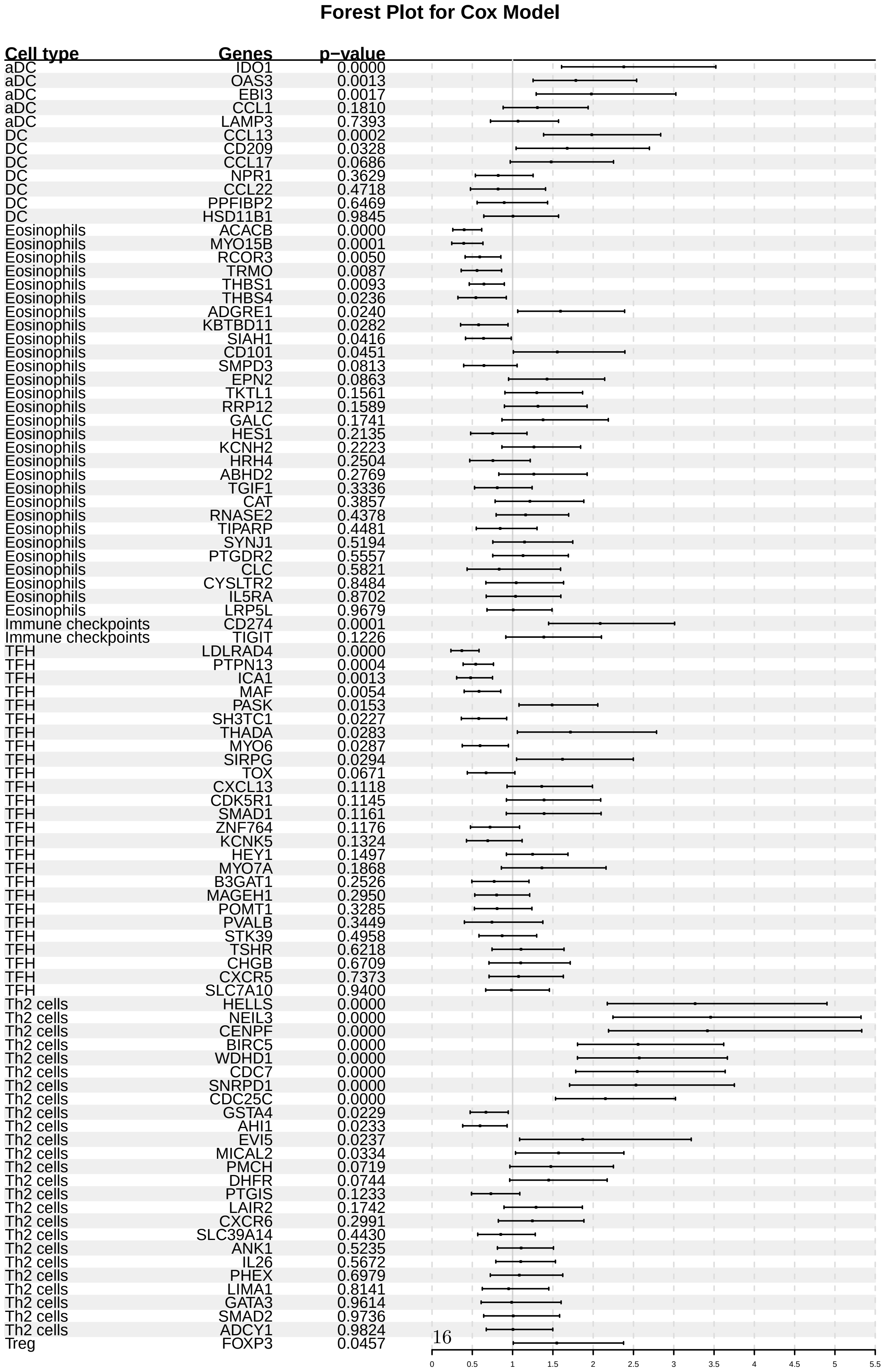
e- Significative immune cells genes survival analysis

Here, we repeat the previous step for the genes of all the significant immune cells.

```
# List of immune cells type significant in coxph analysis
l <- c("DC", "Eosinophils", "Immune checkpoints", "TFH", "Th2 cells",
    "Treg", "aDC")

# Select the genes for cell types selected
df_selected_imm <- df_CTA_immune_whole_clean_avg_82[df_CTA_immune_whole_clean_avg_82$Signature %in%
    l, ] %>%
    select(Signature)
df_selected_imm$Variable <- rownames(df_selected_imm)
df_selected_imm_expr <- df_z_scores_82[rownames(df_z_scores_82) %in%
    rownames(df_selected_imm), -c(1, 2)]
df_selected_imm_expr <- as.data.frame(t(df_selected_imm_expr))
df_selected_imm_expr$Patient <- rownames(df_selected_imm_expr)

# Merge with metadata
df_selected_imm_expr <- merge(df_metadata_surv[, c("Patient",
    "OS.delay", "OS.event")], df_selected_imm_expr, by = "Patient")
rownames(df_selected_imm_expr) <- df_selected_imm_expr$Patient
```

```r
df_selected_imm_expr <- df_selected_imm_expr[, -1]

# Apply coxph model and merge with cel types
results_selected_imm <- apply_coxph_model(df_selected_imm_expr)
results_selected_imm <- merge(results_selected_imm, df_selected_imm,
    by = "Variable")

# Create forest plot
# pdf('../results/figures/forest_plots/all_indiv/forest_plot_selected_imm_82.pdf',
# height = 15, width = 8)
generate_forestplot(results_selected_imm, Type = TRUE)
```

```r
# dev.off()
```



Cell type	Genes	p−value
aDC	IDO1	0.0000
aDC	OAS3	0.0013
aDC	EBI3	0.0017
aDC	CCL1	0.1810
aDC	LAMP3	0.7393
DC	CCL13	0.0002
DC	CD209	0.0328
DC	CCL17	0.0686
DC	NPR1	0.3629
DC	CCL22	0.4718
DC	PPFIBP2	0.6469
DC	HSD11B1	0.9845
Eosinophils	ACACB	0.0000
Eosinophils	MYO15B	0.0001
Eosinophils	RCOR3	0.0050
Eosinophils	TRMO	0.0087
Eosinophils	THBS1	0.0093
Eosinophils	THBS4	0.0236
Eosinophils	ADGRE1	0.0240
Eosinophils	KBTBD11	0.0282
Eosinophils	SIAH1	0.0416
Eosinophils	CD101	0.0451
Eosinophils	SMPD3	0.0813
Eosinophils	EPN2	0.0863
Eosinophils	TKTL1	0.1561
Eosinophils	RRP12	0.1589
Eosinophils	GALC	0.1741
Eosinophils	HES1	0.2135
Eosinophils	KCNH2	0.2223
Eosinophils	HRH4	0.2504
Eosinophils	ABHD2	0.2769
Eosinophils	TGIF1	0.3336
Eosinophils	CAT	0.3857
Eosinophils	RNASE2	0.4378
Eosinophils	TIPARP	0.4481
Eosinophils	SYNJ1	0.5194
Eosinophils	PTGDR2	0.5557
Eosinophils	CLC	0.5821
Eosinophils	CYSLTR2	0.8484
Eosinophils	IL5RA	0.8702
Eosinophils	LRP5L	0.9679
Immune checkpoints	CD274	0.0001
Immune checkpoints	TIGIT	0.1226
TFH	LDLRAD4	0.0000
TFH	PTPN13	0.0004
TFH	ICA1	0.0013
TFH	MAF	0.0054
TFH	PASK	0.0153
TFH	SH3TC1	0.0227
TFH	THADA	0.0283
TFH	MYO6	0.0287
TFH	SIRPG	0.0294
TFH	TOX	0.0671
TFH	CXCL13	0.1118
TFH	CDK5R1	0.1145
TFH	SMAD1	0.1161
TFH	ZNF764	0.1176
TFH	KCNK5	0.1324
TFH	HEY1	0.1497
TFH	MYO7A	0.1868
TFH	B3GAT1	0.2526
TFH	MAGEH1	0.2950
TFH	POMT1	0.3285
TFH	PVALB	0.3449
TFH	STK39	0.4958
TFH	TSHR	0.6218
TFH	CHGB	0.6709
TFH	CXCR5	0.7373
TFH	SLC7A10	0.9400
Th2 cells	HELLS	0.0000
Th2 cells	NEIL3	0.0000
Th2 cells	CENPF	0.0000
Th2 cells	BIRC5	0.0000
Th2 cells	WDHD1	0.0000
Th2 cells	CDC7	0.0000
Th2 cells	SNRPD1	0.0000
Th2 cells	CDC25C	0.0000
Th2 cells	GSTA4	0.0229
Th2 cells	AHI1	0.0233
Th2 cells	EVI5	0.0237
Th2 cells	MICAL2	0.0334
Th2 cells	PMCH	0.0719
Th2 cells	DHFR	0.0744
Th2 cells	PTGIS	0.1233
Th2 cells	LAIR2	0.1742
Th2 cells	CXCR6	0.2991
Th2 cells	SLC39A14	0.4430
Th2 cells	ANK1	0.5235
Th2 cells	IL26	0.5672
Th2 cells	PHEX	0.6979
Th2 cells	LIMA1	0.8141
Th2 cells	GATA3	0.9614
Th2 cells	SMAD2	0.9736
Th2 cells	ADCY1	0.9824
Treg	FOXP3	0.0457

Figure 5: Forest plot for significative immune cells (n = 82)

This forest plot show the impact of each cell types on the survival probabilities. We can see the genes that are drive the results.

2) Conventional chondrosarcomas

This section concern the survival analysis for conventional chondrosarcomas. This is the same stpes than previously.

a- Categorical data

```
# Compute medians for each immune cell types
medians_sign <- apply(df_imm_z_scores_63, 2, median)

# Adding categorical column
df_cat_conv <- df_imm_z_scores_63
for (col in colnames(df_cat_conv)) {
    new_column_name <- paste(col, "cat", sep = " ")
    df_cat_conv[[new_column_name]] <- ifelse(df_imm_z_scores_63[[col]] >
        medians_sign[col], "HIGH", "LOW")
}

# Select categories
df_cat_conv <- df_cat_conv[, grep("cat", colnames(df_cat_conv))]

# Replace space by _
colnames(df_cat_conv) <- gsub(" ", "_", colnames(df_cat_conv))

# Merge df
df_cat_conv$Patient <- rownames(df_cat_conv)
df_survival_conv_cat <- merge(df_metadata_surv_conv, df_cat_conv,
    by = "Patient")
rownames(df_survival_conv_cat) <- df_survival_conv_cat$Patient
df_survival_conv_cat <- df_survival_conv_cat[, -1]

# Apply coxph model and create forest plot
results <- apply_coxph_model(df_survival_conv_cat)
# write.table(results, '../results/results_coxhp.tsv', sep
# = '\t', row.names = FALSE, quote = FALSE)
generate_forestplot(results, Type = FALSE)
```

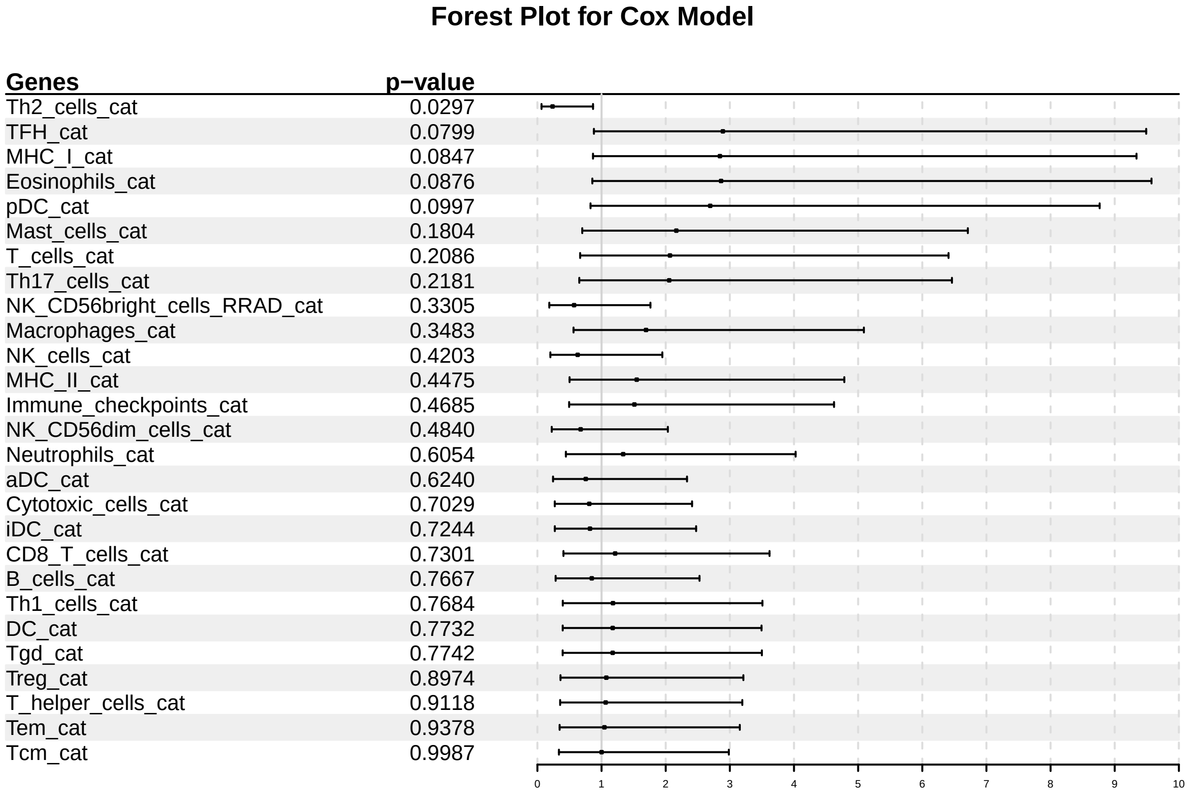


Figure 6: Forest plot for categorical immune cell types expression (n = 63)

By using categorical data and Cox model, there is only Th2 that are signnificant.

b- Continuous data

```
df_cont_conv <- df_imm_z_scores_63

# Replace space by _
colnames(df_cont_conv) <- gsub(" ", "_", colnames(df_cont_conv))

# Merge df
df_cont_conv$Patient <- rownames(df_cont_conv)
df_survival_conv <- merge(df_metadata_surv_conv, df_cont_conv,
    by = "Patient")
rownames(df_survival_conv) <- df_survival_conv$Patient
df_survival_conv <- df_survival_conv[, -1]

# Apply coxph model and generate plot
results_cont <- apply_coxph_model(df_survival_conv)
# write.table(results_cont,
# '../results/results_coxph_var_cont_patients_conv.tsv',
# sep = '\t', row.names = FALSE, quote = FALSE)
# pdf('../results/figures/forest_plots/conv_chondro/forest_plot_conv_patients_var_cont.pdf')
generate_forestplot(results_cont, Type = FALSE)
```

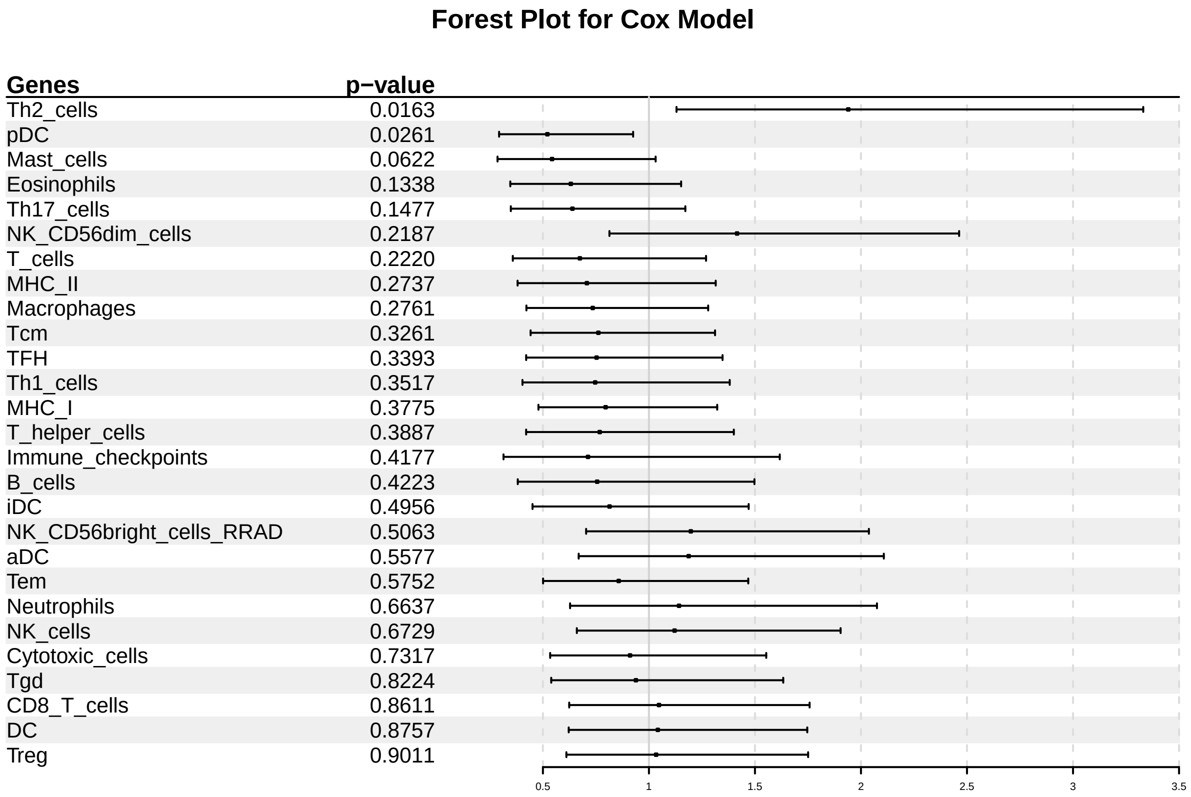


Figure 7: Forest plot for continuous immune cell types expression (n = 63)

```
# dev.off()
```

By using z-scores, pDC are now significant and have a good impact on survival probabilities.

c- Kaplan-Meier plots

This shows the graph for each significant HR ratio more than significant to all patients to observe the differences.

```r
# Create Kaplan-Meier plots with categorical data
p1 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ DC_cat, data = df_survival_conv_cat),
    df_survival_conv_cat)
p2 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Eosinophils_cat,
    data = df_survival_conv_cat), df_survival_conv_cat)
p3 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Immune_checkpoints_cat,
    data = df_survival_conv_cat), df_survival_conv_cat)
p4 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ TFH_cat, data = df_survival_conv_cat),
    df_survival_conv_cat)
p5 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Th2_cells_cat,
    data = df_survival_conv_cat), df_survival_conv_cat)
p6 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Treg_cat, data = df_survival_conv_cat),
    df_survival_conv_cat)
p7 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ aDC_cat, data = df_survival_conv_cat),
    df_survival_conv_cat)
p8 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ pDC_cat, data = df_survival_conv_cat),
    df_survival_conv_cat)
# pdf('../results/figures/other_plots/KM_plots_signif_cox_conv_indiv.pdf',
# height = 20, width = 15)
grid.arrange(p1, p2, p3, p4, p5, p6, p7, p8, ncol = 2)
```

```r
# dev.off()
```

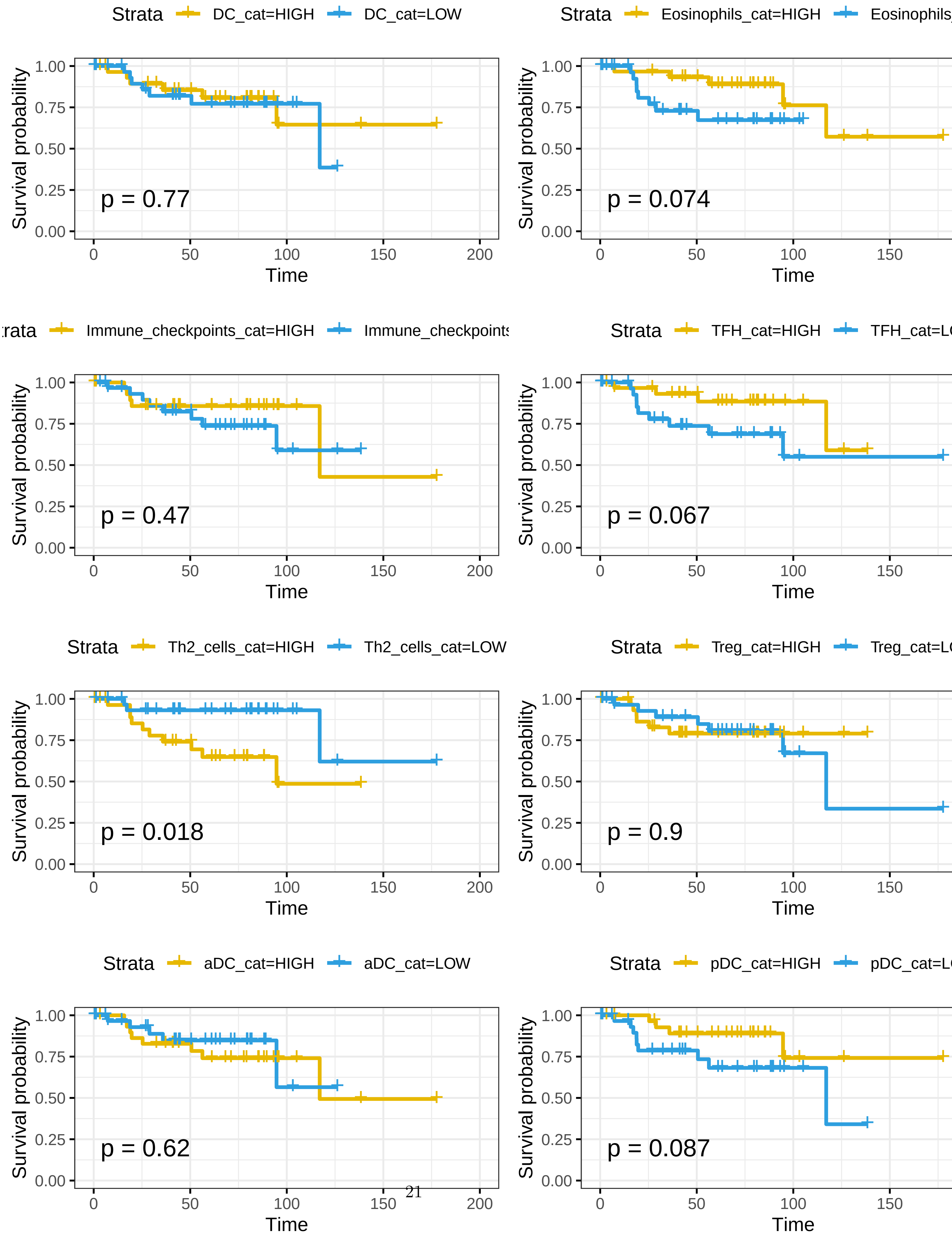


Figure 8: Kaplan-Meier plots for immune cell types (n = 63)

With this test, we see that only Th2 cells are significant. So we see that this type may be play an important role in the survival.

d- Survival analysis for Th2 cells

```r
# Take conventional tumors
df_survival_th2 <- merge(df_metadata_surv_conv, df_expr_th2,
    by = "Patient")
rownames(df_survival_th2) <- df_survival_th2$Patient
df_survival_th2 <- df_survival_th2[, -1]

# Apply coxph model and create forest plot
results_th2 <- apply_coxph_model(df_survival_th2)
# pdf('../results/figures/forest_plots/conv_chondro/forest_plot_conv_th2.pdf')
generate_forestplot(results_th2, Type = FALSE)
```

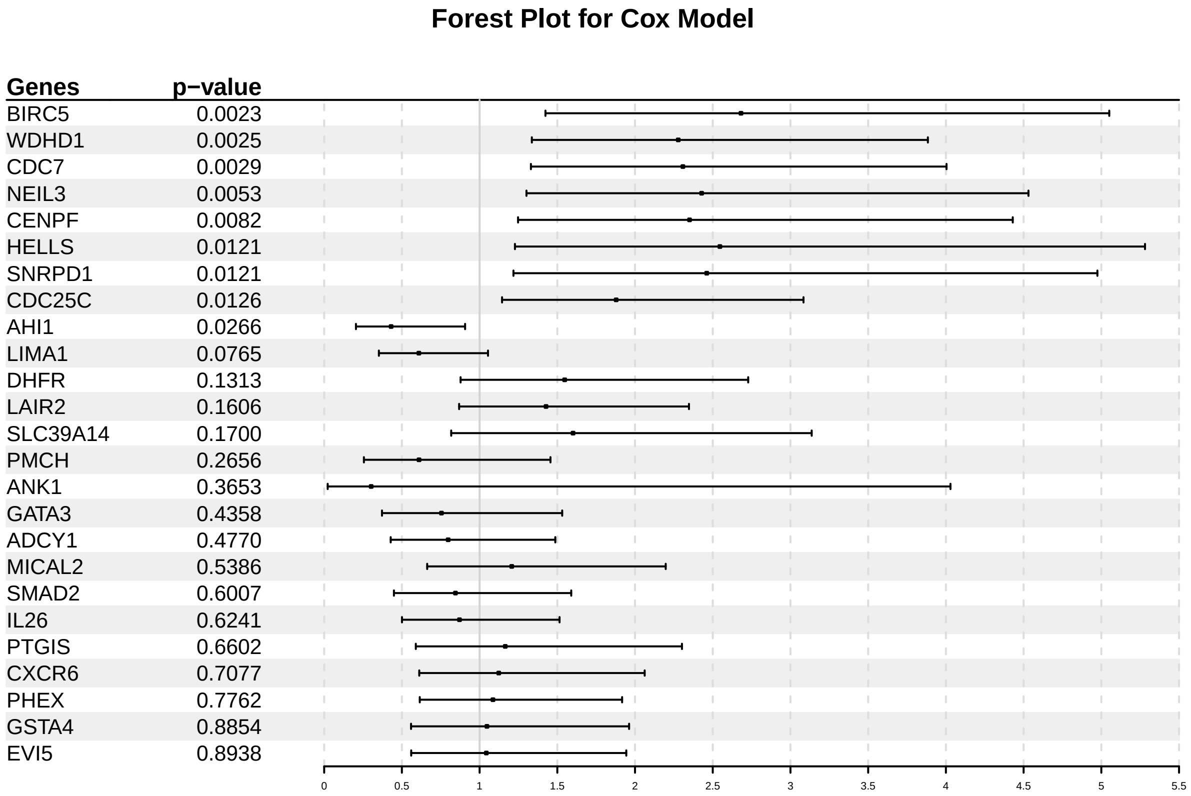


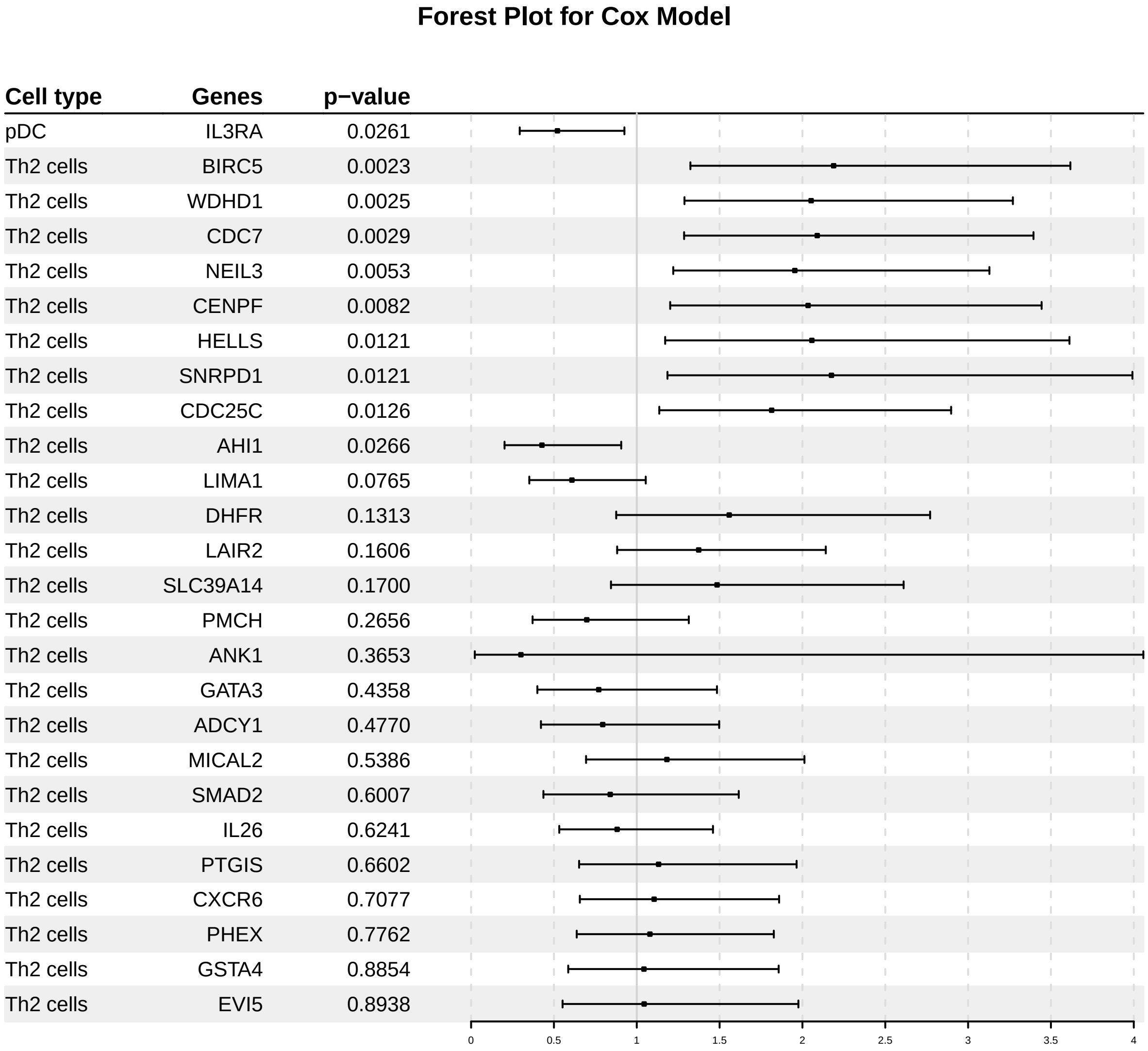
Figure 9: Forest plot for Th2 cells (n = 63)

```r
# dev.off()
```

e- Significative immune cells genes survival analysis

```r
# List of immune cell types significant
l_th2_pdc <- c("Th2 cells", "pDC")

# Select the genes for cell types selected
df_selected_imm <- df_CTA_immune_whole_clean_avg_63[df_CTA_immune_whole_clean_avg_63$Signature %in%
    l_th2_pdc, ] %>%
    select(Signature)
df_selected_imm$Variable <- rownames(df_selected_imm)
df_selected_imm_expr_conv <- df_z_scores_63[rownames(df_z_scores_63) %in%
    rownames(df_selected_imm), -c(1, 2)]
df_selected_imm_expr_conv <- as.data.frame(t(df_selected_imm_expr_conv))
df_selected_imm_expr_conv$Patient <- rownames(df_selected_imm_expr_conv)

# Merge with metadata
df_selected_imm_expr_conv <- merge(df_metadata_surv_conv[, c("Patient",
    "OS.delay", "OS.event")], df_selected_imm_expr_conv, by = "Patient")
rownames(df_selected_imm_expr_conv) <- df_selected_imm_expr_conv$Patient
df_selected_imm_expr_conv <- df_selected_imm_expr_conv[, -1]

# Apply coxph model and create forest plot
results_selected_imm_conv <- apply_coxph_model(df_selected_imm_expr_conv)
results_selected_imm_conv <- merge(results_selected_imm_conv,
    df_selected_imm, by = "Variable")
results_selected_imm_conv <- results_selected_imm_conv[order(results_selected_imm_conv$Signature,
    results_selected_imm_conv$Pvalue), ]
# pdf('../results/figures/forest_plots/conv_chondro/forest_plot_selected_imm_conv_indiv.pdf',
# height = 8, width = 8)
generate_forestplot(results_selected_imm_conv, Type = TRUE)
```

```r
# dev.off()
```



Cell type	Genes	p−value
pDC	IL3RA	0.0261
Th2 cells	BIRC5	0.0023
Th2 cells	WDHD1	0.0025
Th2 cells	CDC7	0.0029
Th2 cells	NEIL3	0.0053
Th2 cells	CENPF	0.0082
Th2 cells	HELLS	0.0121
Th2 cells	SNRPD1	0.0121
Th2 cells	CDC25C	0.0126
Th2 cells	AHI1	0.0266
Th2 cells	LIMA1	0.0765
Th2 cells	DHFR	0.1313
Th2 cells	LAIR2	0.1606
Th2 cells	SLC39A14	0.1700
Th2 cells	PMCH	0.2656
Th2 cells	ANK1	0.3653
Th2 cells	GATA3	0.4358
Th2 cells	ADCY1	0.4770
Th2 cells	MICAL2	0.5386
Th2 cells	SMAD2	0.6007
Th2 cells	IL26	0.6241
Th2 cells	PTGIS	0.6602
Th2 cells	CXCR6	0.7077
Th2 cells	PHEX	0.7762
Th2 cells	GSTA4	0.8854
Th2 cells	EVI5	0.8938

Figure 10: Forest plot for significative immune cells (n = 63)

II. CTA survival analysis

This section analyzes the impact of CTA on survival probabilites.

1) All chondrosarcoma types

This part concern all the patients.

```r
# CTA data
df_expr_cta_82 <- subset(df_z_scores_82, CTA == "CTA")
df_expr_cta_82 <- df_expr_cta_82[, !colnames(df_expr_cta_82) %in%
    c("Signature", "CTA", "SYMBOL")]
df_expr_cta_82 <- as.data.frame(t(df_expr_cta_82))
df_expr_cta_82$Patient <- rownames(df_expr_cta_82)
df_expr_cta_82 <- merge(df_metadata_surv, df_expr_cta_82, by = "Patient")
rownames(df_expr_cta_82) <- df_expr_cta_82$Patient
df_expr_cta_82 <- df_expr_cta_82[, -1]

res_cta <- apply_coxph_model(df_expr_cta_82)
# write.table(res_cta,
# '../results/results_coxph_cta_all_indiv.tsv', sep = '\t',
# row.names = FALSE, quote = FALSE)

# Forest plot on significative CTA
res_cta_signif <- res_cta[res_cta$Pvalue < 0.05, ]
# pdf('../results/figures/forest_plots/all_indiv/forest_plot__var_cont_cta_zscores_82.pdf',
# width = 10, height = 30)
generate_forestplot(res_cta_signif, Type = FALSE)
```

```r
# dev.off()
```

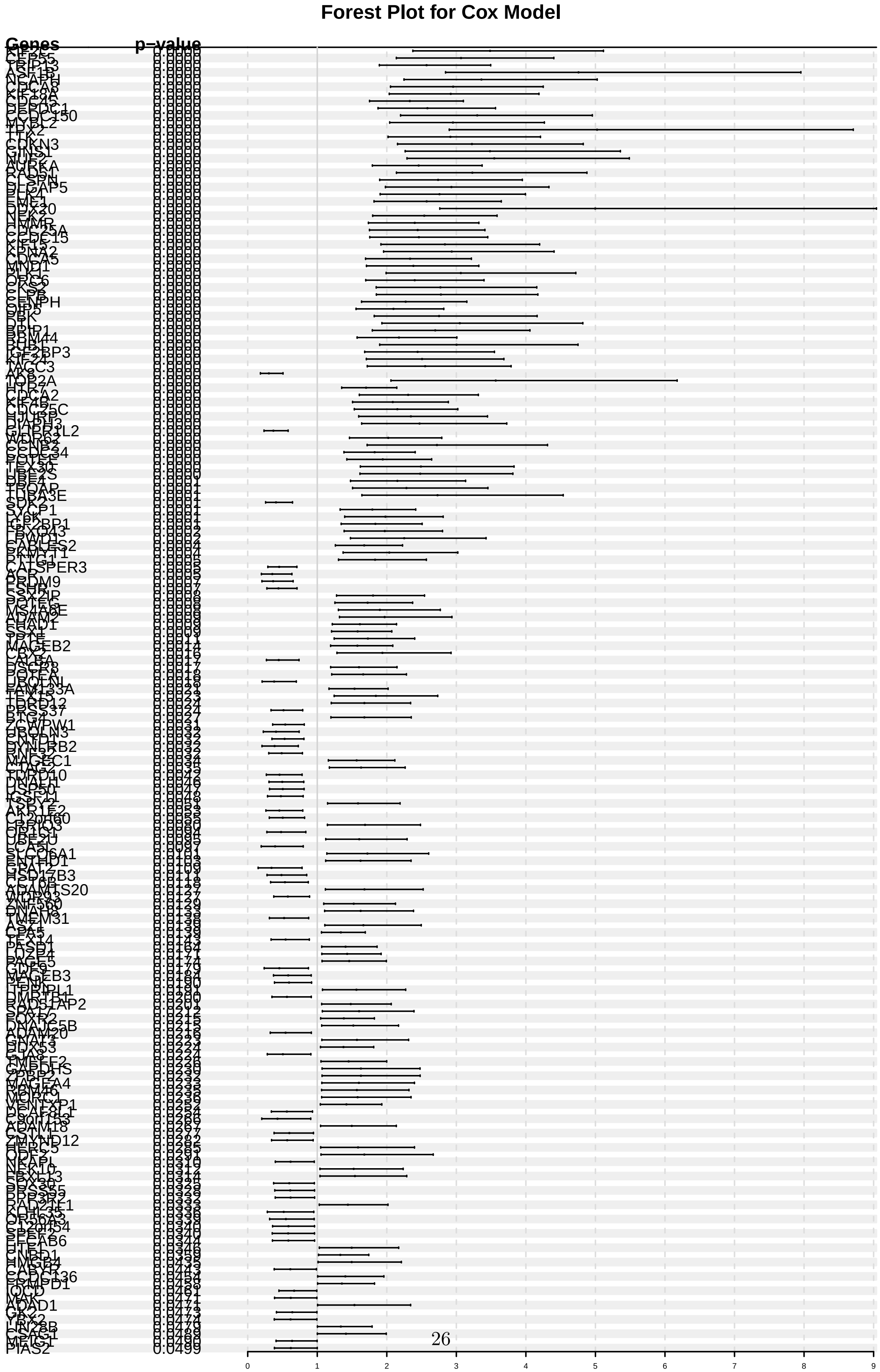


Figure 11: Forest plot for significant CTA (n = 82)

This forest plot illustrates only the significant HR. We see here that there is more bad impact CTA than good impact. We uses this CTA list in script 5 to analyze expression and make a clustering. This results illustrates that there is groups so we make survival analysis to see the difference between group (see III.)

a- Intensities jitter plot for significant CTA

In this section, we want to see the intensities per significant CTA to visualize.

```r
# Read intensities
df <- df_CTA_immune_whole_clean_avg_82[, -c(1, 2)]
df <- df[rownames(df) %in% res_cta_signif[, 1], ]
df$SYMBOL <- rownames(df)

# Cluster data
cluster_data <- read.table("../results/clusters_indiv/clusters_cta_signif_coxph_all_indiv.tsv",
    sep = "\t", header = TRUE)
cluster_data$Cluster <- as.character(cluster_data$Cluster)

# Data transformation for the plot
df_long <- pivot_longer(df, cols = -SYMBOL, values_to = "value")

# Merge
df_long <- pivot_longer(df, cols = -SYMBOL, values_to = "value")
df_long <- merge(df_long, cluster_data, by = "SYMBOL")

# pdf(file =
# '../results/figures/other_plots/plot_cta_coxph_signif_all_indiv.pdf',
# height = 10, width = 50)
ggplot(df_long, aes(x = reorder(SYMBOL, -value), y = value, color = as.factor(Cluster))) +
    geom_jitter(width = 0.2, height = 0, size = 1, alpha = 0.3) +
    geom_boxplot(alpha = 0.4, outliers = FALSE, colour = "black",
        fill = NA) + labs(title = "Plot of intensities per significant coxph CTA",
    x = "CTA", y = "Intensities") + scale_color_manual(values = c(`1` = "#EA4343",
    `2` = "blue", `3` = "#75E05A")) + theme(axis.text.x = element_text(angle = 90,
    hjust = 1))
```

```r
# dev.off()
```

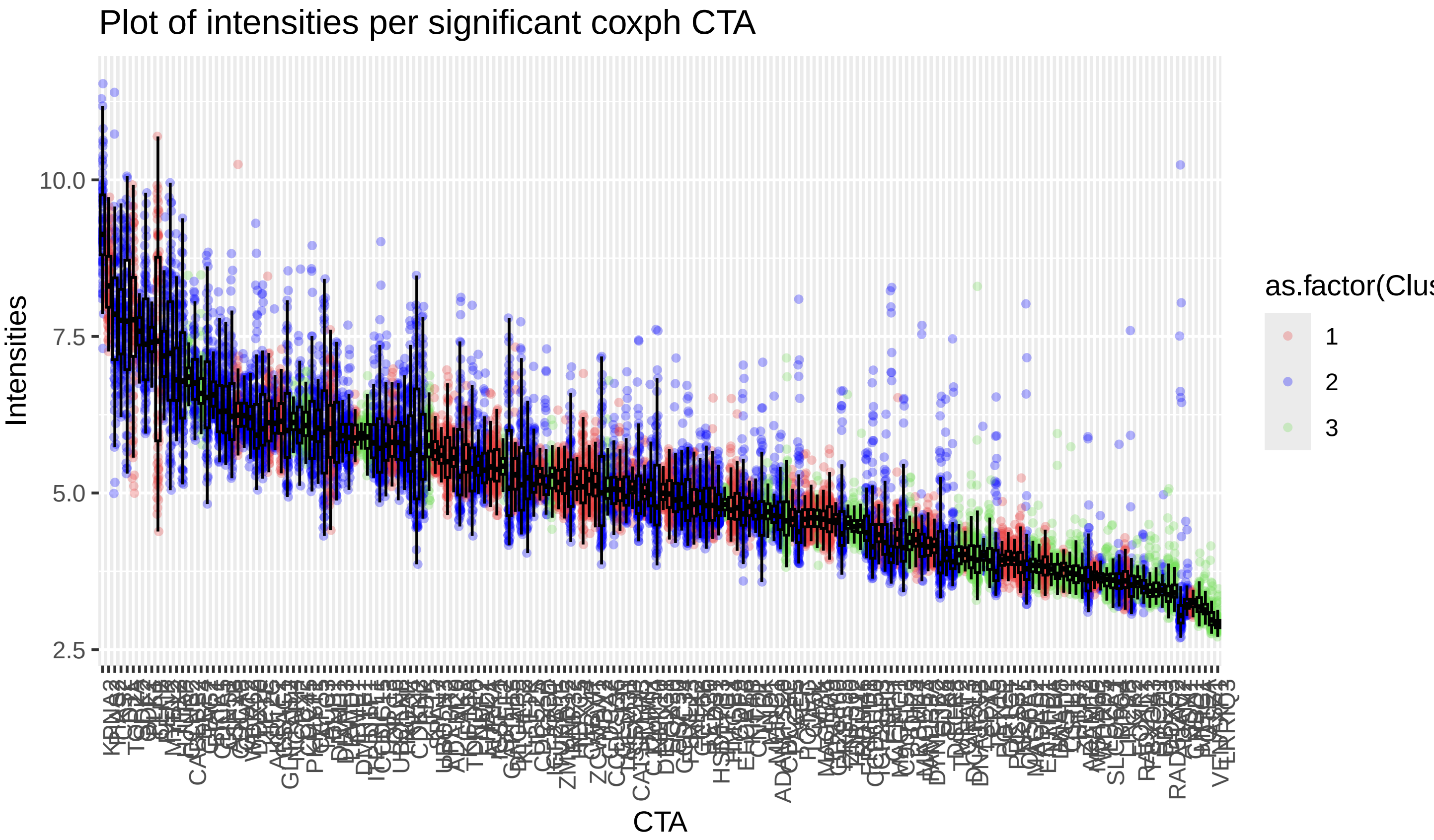


Figure 12: Intensitites jitter plot for significant CTA (n = 82)

b- Normalized intensities for CTA to see the differences

Here, it is a test of survival analysis with normalized data and not z-scores to see if there are differences.

```
df_int <- df_CTA_immune_whole_clean_avg_82
df_int <- subset(df_int, CTA == "CTA")
df_int <- df_int[, !colnames(df_int) %in% c("Signature", "CTA",
    "SYMBOL")]
df_int <- as.data.frame(t(df_int))
df_int <- df_int[df_metadata_surv$Patient, ]
df_int$Patient <- rownames(df_int)
df_survival_int_cta <- merge(df_metadata_surv, df_int, by = "Patient")
rownames(df_survival_int_cta) <- df_survival_int_cta$Patient
df_survival_int_cta <- df_survival_int_cta[, -1]
res_cta_int <- apply_coxph_model(df_survival_int_cta)
res_cta_signif_int <- res_cta_int[res_cta_int$Pvalue < 0.05,
    ]
head(res_cta_signif_int)
```

```
##    Variable          HR     LowerCI    UpperCI       Pvalue
## 4       ACR  0.01276281 0.001090762  0.1493354 0.0005104523
## 14    ADAD1  7.75991984 1.026307431 58.6728246 0.0471308847
## 16   ADAM18  6.51130221 1.241794511 34.1417651 0.0266833243
## 17    ADAM2 15.85474040 3.102621206 81.0194917 0.0008989017
## 18   ADAM20  0.29768624 0.105880426  0.8369545 0.0215944351
## 22 ADAMTS20  4.33091460 1.367858121 13.7125489 0.0126789718
```

We see that with the intensities data that the p-val are the same as continuous data (in z-scores) but the HR are less clean than z score data.

2) Conventional chondrosarcomas

This section like part I concerns conventional chondrosarcomas

```
# CTA data CTA data
df_expr_cta_63 <- subset(df_z_scores_63, CTA == "CTA")
df_expr_cta_63 <- df_expr_cta_63[, !colnames(df_expr_cta_63) %in%
    c("Signature", "CTA", "SYMBOL")]
df_expr_cta_63 <- as.data.frame(t(df_expr_cta_63))
df_expr_cta_63$Patient <- rownames(df_expr_cta_63)
df_expr_cta_63 <- merge(df_metadata_surv, df_expr_cta_63, by = "Patient")
rownames(df_expr_cta_63) <- df_expr_cta_63$Patient
df_expr_cta_63 <- df_expr_cta_63[, -1]

# Apply coxph and generate forest plot with significant
# data
res_cta <- apply_coxph_model(df_expr_cta_63)
# write.table(res_cta,
# '../results/results_coxph_cta_conv_indiv.tsv', sep =
# '\t', row.names = FALSE, quote = FALSE)

res_cta_signif <- res_cta[res_cta$Pvalue < 0.05, ]
```

```
# pdf('../results/figures/forest_plots/conv_chondro/forest_plot_var_cont_cta_zscores_63.pdf',
# width = 10, height = 30)
generate_forestplot(res_cta_signif, Type = FALSE)
```

```
# dev.off()
```

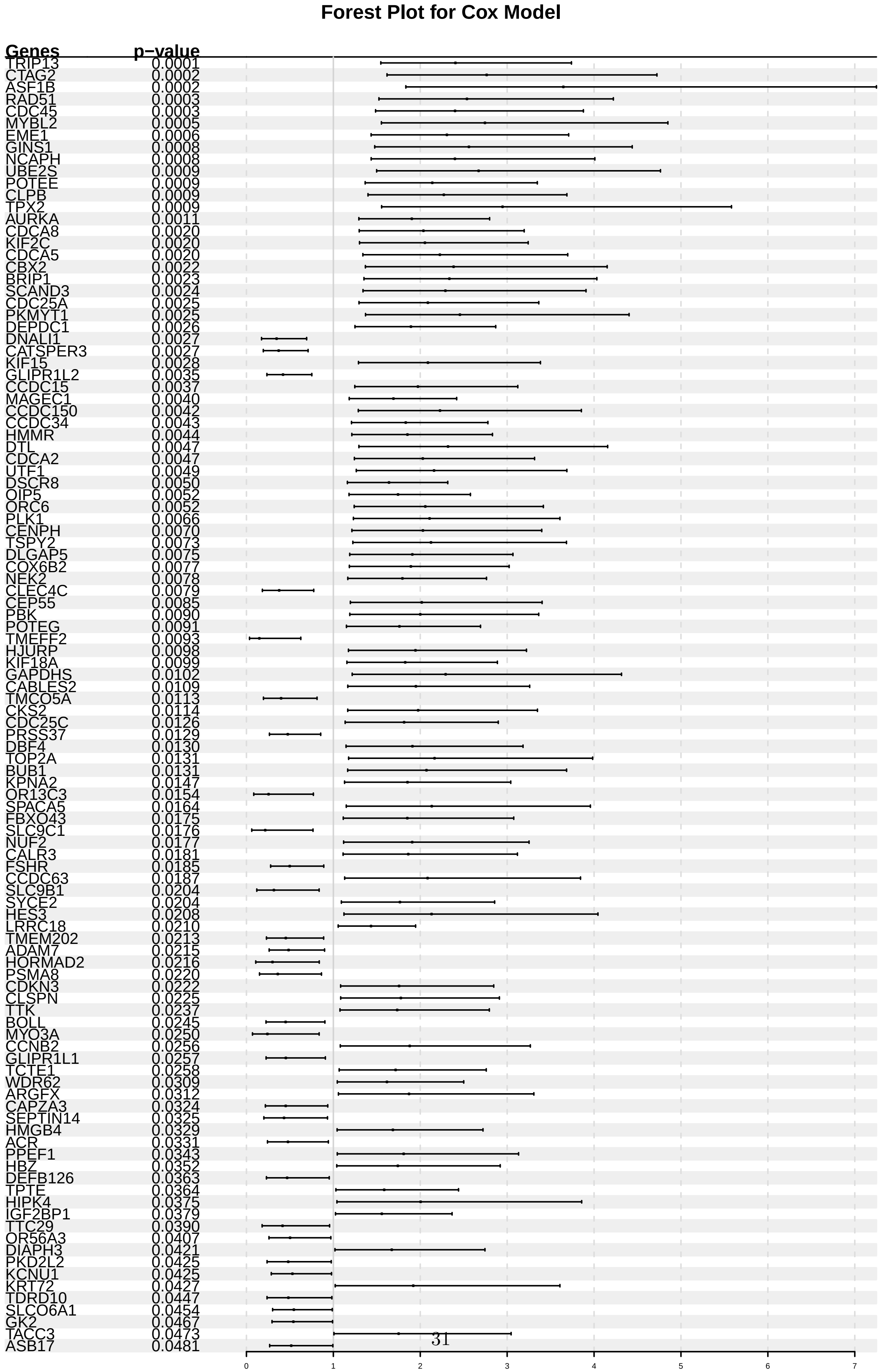


Figure 13: Forest plot for significant CTA (n = 63)

Same than previously, we see that there is more bad impact CTA than good. With this, we have also clustering these CTA to see groups and make another analysis.

a- Itensities jitter plot for significant CTA

```r
# Prepare data
df <- df_CTA_immune_whole_clean_avg_63[, -c(1, 2)]
df <- df[rownames(df) %in% res_cta_signif[, 1], ]
df$SYMBOL <- rownames(df)

# Cluster data
cluster_data <- read.table("../results/clusters_indiv/clusters_cta_signif_conv_indiv.tsv",
    sep = "\t", header = TRUE)
cluster_data$Cluster <- as.character(cluster_data$Cluster)

# Data transformation for the plot
df_long <- pivot_longer(df, cols = -SYMBOL, values_to = "value")

# Merge
df_long <- pivot_longer(df, cols = -SYMBOL, values_to = "value")
df_long <- merge(df_long, cluster_data, by = "SYMBOL")

# pdf(file =
# '../results/figures/other_plots/plot_cta_coxph_signif_conv_indiv.pdf',
# height = 10, width = 20)
ggplot(df_long, aes(x = reorder(SYMBOL, -value), y = value, color = as.factor(Cluster))) +
    geom_jitter(width = 0.2, height = 0, size = 1, alpha = 0.3) +
    geom_boxplot(alpha = 0.4, outliers = FALSE, colour = "black",
        fill = NA) + labs(title = "Plot of intensities per significant coxph CTA",
    x = "CTA", y = "Intensities") + scale_color_manual(values = c(`1` = "#EA4343",
    `2` = "blue", `3` = "#75E05A")) + theme(axis.text.x = element_text(angle = 90,
    hjust = 1))
```

```r
# dev.off()
```

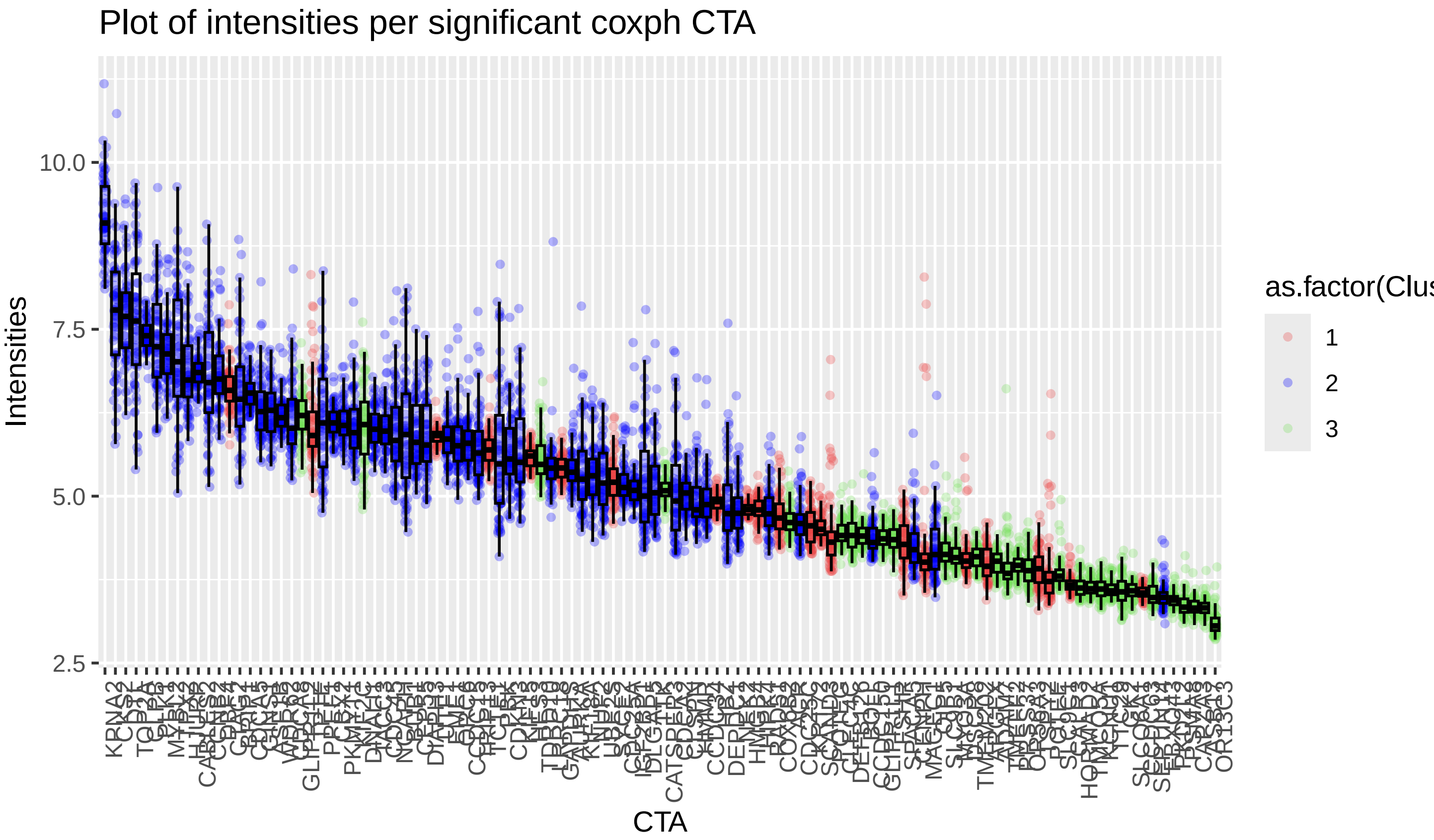


Figure 14: Intensitites jitter plot for significant CTA (n = 63)

b- KM plots selected CTA

```r
df_expr_cta_63_select <- df_expr_cta_63[, c("OS.delay", "OS.event",
    "DTL", "HJURP", "GINS1", "PBK", "CTAG2", "CT45A1", "CSAG1",
    "PRAME")]

genes <- colnames(df_expr_cta_63_select)[-(1:2)]

# list of cutoff
cut_list <- lapply(genes, function(gene) {
    cut <- surv_cutpoint(df_expr_cta_63_select, time = "OS.delay",
        event = "OS.event", variables = gene, minprop = 0.3)
    data.frame(gene = gene, cutpoint = summary(cut)$cutpoint[[1]],
        stringsAsFactors = FALSE)
})

# Combine
cutpoints_df <- do.call(rbind, cut_list)

# Add row
df_cat_cta_63_select <- df_expr_cta_63_select

for (col in colnames(df_cat_cta_63_select[, -c(1, 2)])) {
    threshold <- cutpoints_df$cutpoint[cutpoints_df$gene == col]
    new_column_name <- paste0(col, "_cat")
    df_cat_cta_63_select[[new_column_name]] <- ifelse(df_expr_cta_63_select[[col]] >
        threshold, "HIGH", "LOW")
}

# Replace space by _
colnames(df_cat_cta_63_select) <- gsub(" ", "_", colnames(df_cat_cta_63_select))
df_cat_cta_63_select$OS.delay <- df_expr_cta_63_select$OS.delay
df_cat_cta_63_select$OS.event <- df_expr_cta_63_select$OS.event

# Create Kaplan-Meier plots with categorical data
p1 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ DTL_cat, data = df_cat_cta_63_select),
    df_cat_cta_63_select)
p2 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ HJURP_cat, data = df_cat_cta_63_select),
    df_cat_cta_63_select)
p3 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ GINS1_cat, data = df_cat_cta_63_select),
    df_cat_cta_63_select)
p4 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ PBK_cat, data = df_cat_cta_63_select),
    df_cat_cta_63_select)
p5 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ CTAG2_cat, data = df_cat_cta_63_select),
    df_cat_cta_63_select)
p6 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ CT45A1_cat,
    data = df_cat_cta_63_select), df_cat_cta_63_select)
p7 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ CSAG1_cat, data = df_cat_cta_63_select),
    df_cat_cta_63_select)
p8 <- plot_km(survfit(Surv(OS.delay, OS.event) ~ PRAME_cat, data = df_cat_cta_63_select),
    df_cat_cta_63_select)

# pdf('../results/figures/other_plots/KM_plots_select_scatter_cta.pdf',
# height = 20, width = 15)
grid.arrange(p1, p2, p3, p4, p5, p6, p7, p8, ncol = 2)
```

```r
# dev.off()
```

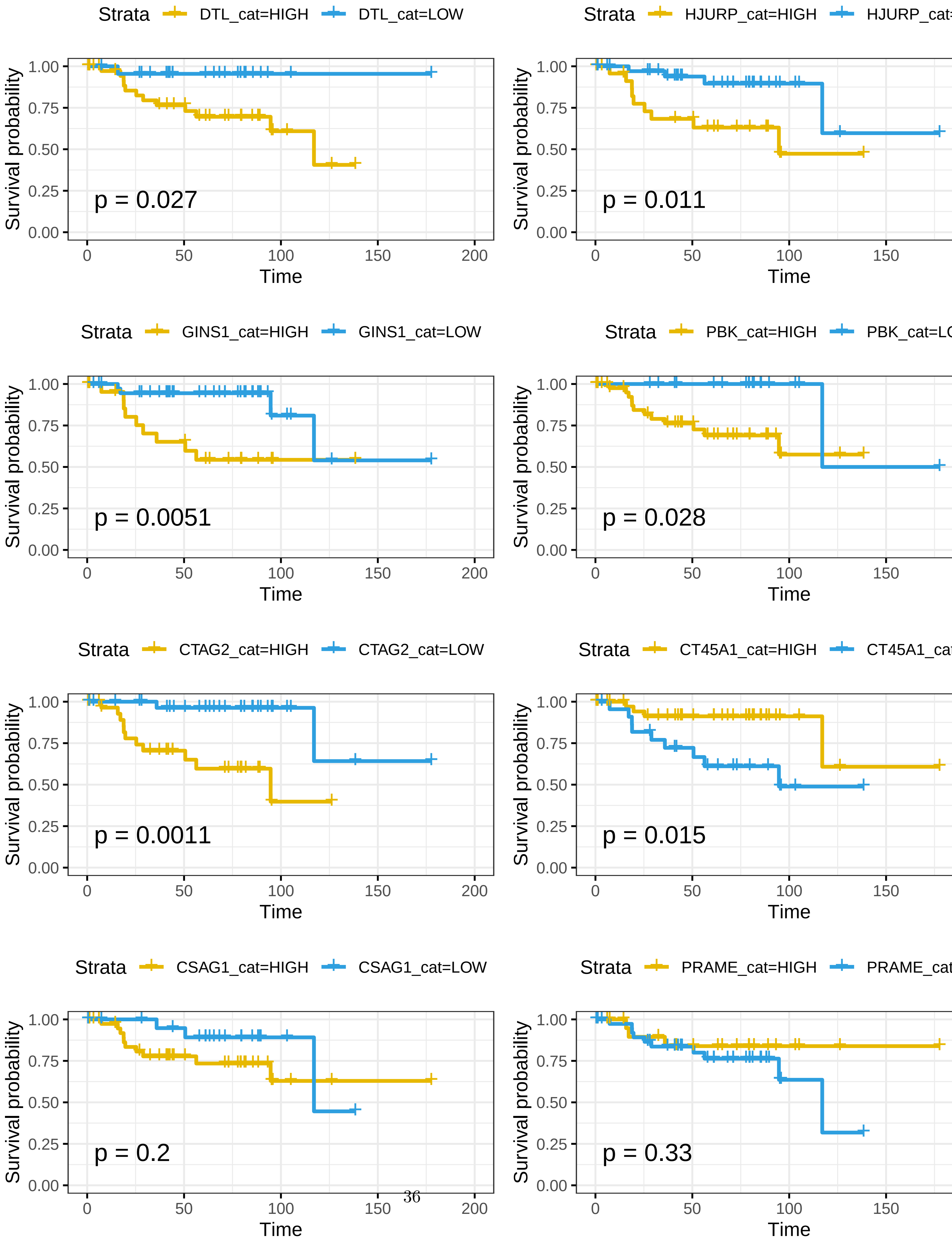


Figure 15: Kaplan-Meier plots for interested CTA (n = 63)

Here, we want to see and illustrate the survival probabilities of interested CTA targets.

III. Survival analysis for clusters from CTA heatmaps

This section use clustering results from script 5 on expression analysis.

1) All chondrosarcoma types

This part compute with all the patients.

a- Clustering Kmeans k = 4 with all chondrosarcoma types

The clustering corresponds to the figure 32.

```
# Read clusters
l_anno_all <- read.table("../results/clusters_indiv/clusters_conv_indiv_mhc.tsv",
    header = TRUE, sep = "\t")
df_cluster_all <- merge(l_anno_all, df_metadata_surv, by = "Patient")
rownames(df_cluster_all) <- df_cluster_all$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_all),
    df_cluster_all)
p
```

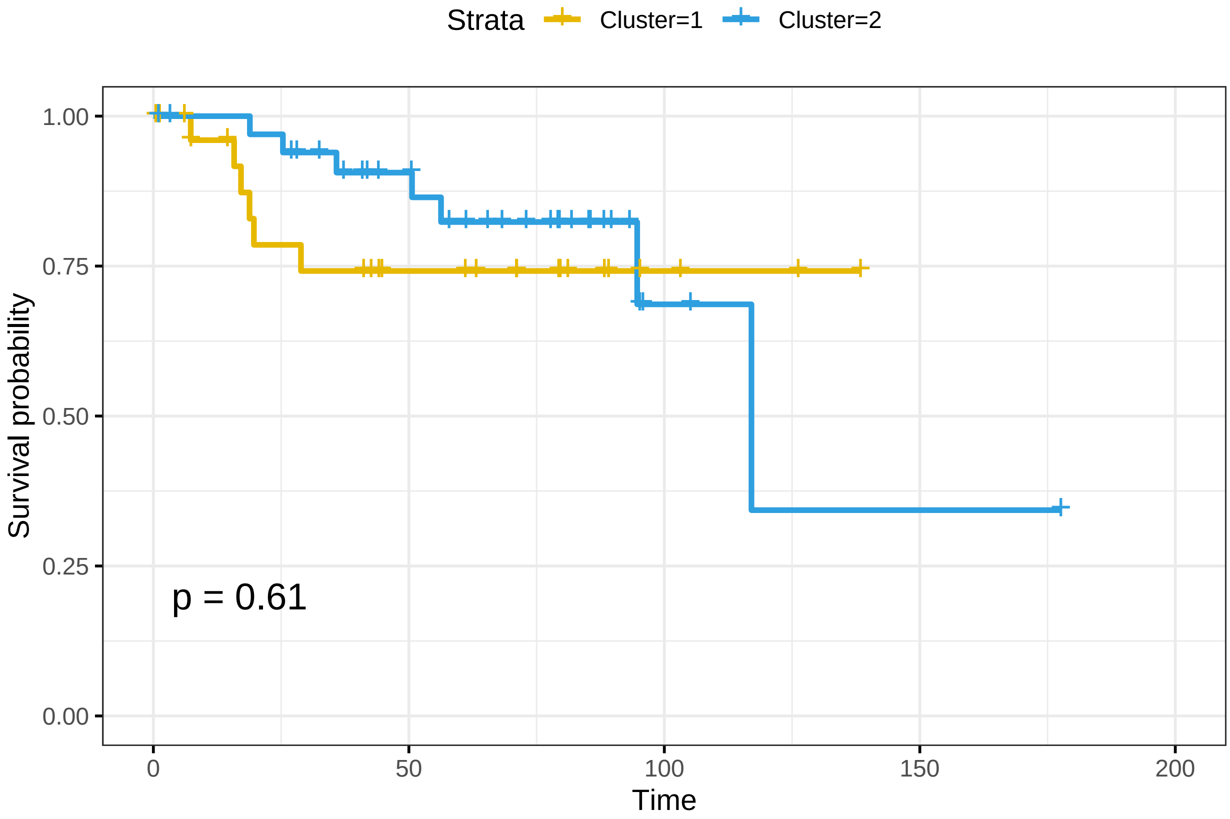


Figure 16: Kaplan-Meier plot with clusters from figure 31 (script 5) (n = 82)

We see that there is no significant differences between the 4 clusters.

b- Significative coxph CTA genes clustering

This clustering is from heatmap 2.

```
# Read clusters
l_cta_all <- read.table("../results/clusters_indiv/clusters_all_indiv_mhc_cta_signif_coxph.tsv",
    header = TRUE, sep = "\t")

df_cluster_cta_all <- merge(l_cta_all, df_metadata_surv, by = "Patient")
rownames(df_cluster_cta_all) <- df_cluster_cta_all$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_cta_all),
    df_cluster_cta_all)
p
```

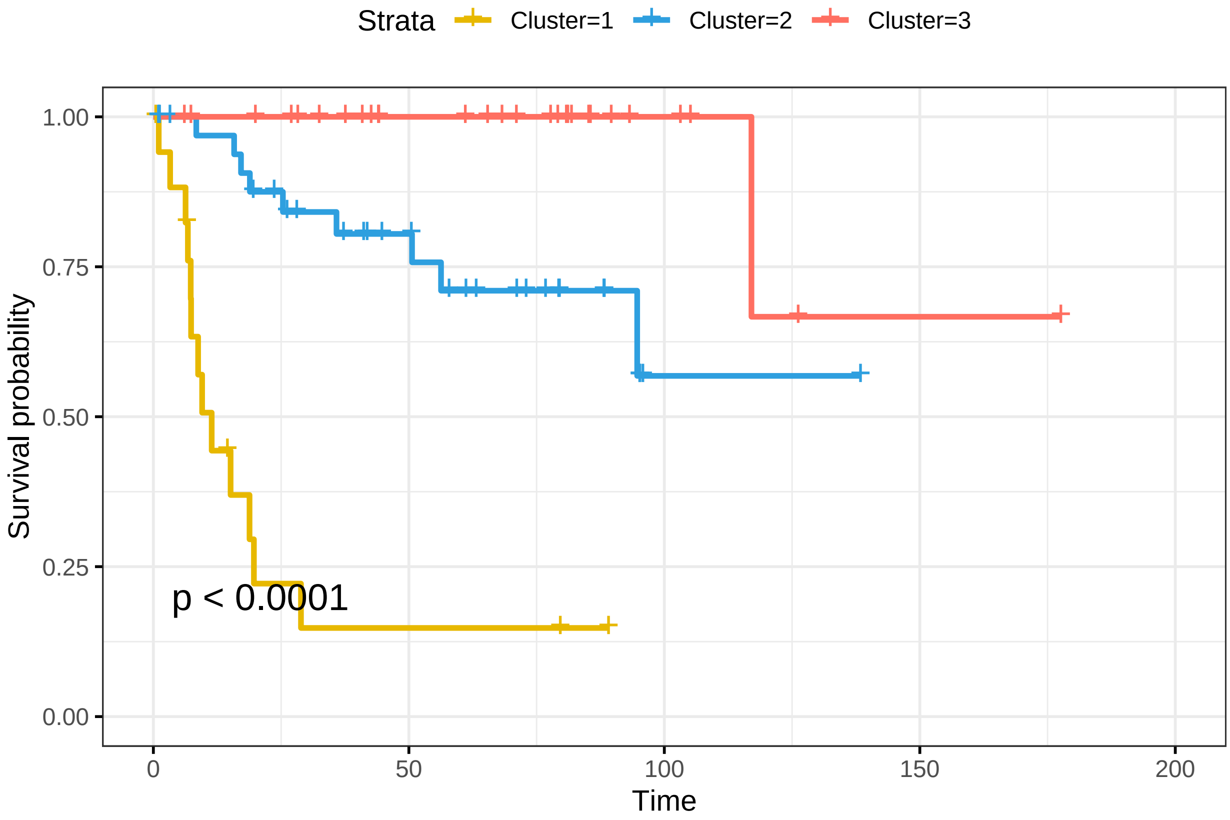


Figure 17: Kaplan-Meier plot with clusters from figure 3 (script 5) (n = 82)

Here we see that the C3 have bad survival and this cluster correspond to an over expression of some CTA. So, we can sat that these CTA could be markers.

c- Selected significant coxph CTA genes clustering

```
# Prepare table
l <- read.table("../results/clusters_indiv/clusters_indiv_signif_selected_cta_all.tsv",
    sep = "\t", header = TRUE)

df_cluster_all <- merge(l, df_metadata_surv, by = "Patient")
rownames(df_cluster_all) <- df_cluster_all$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_all),
    df_cluster_all)
p
```

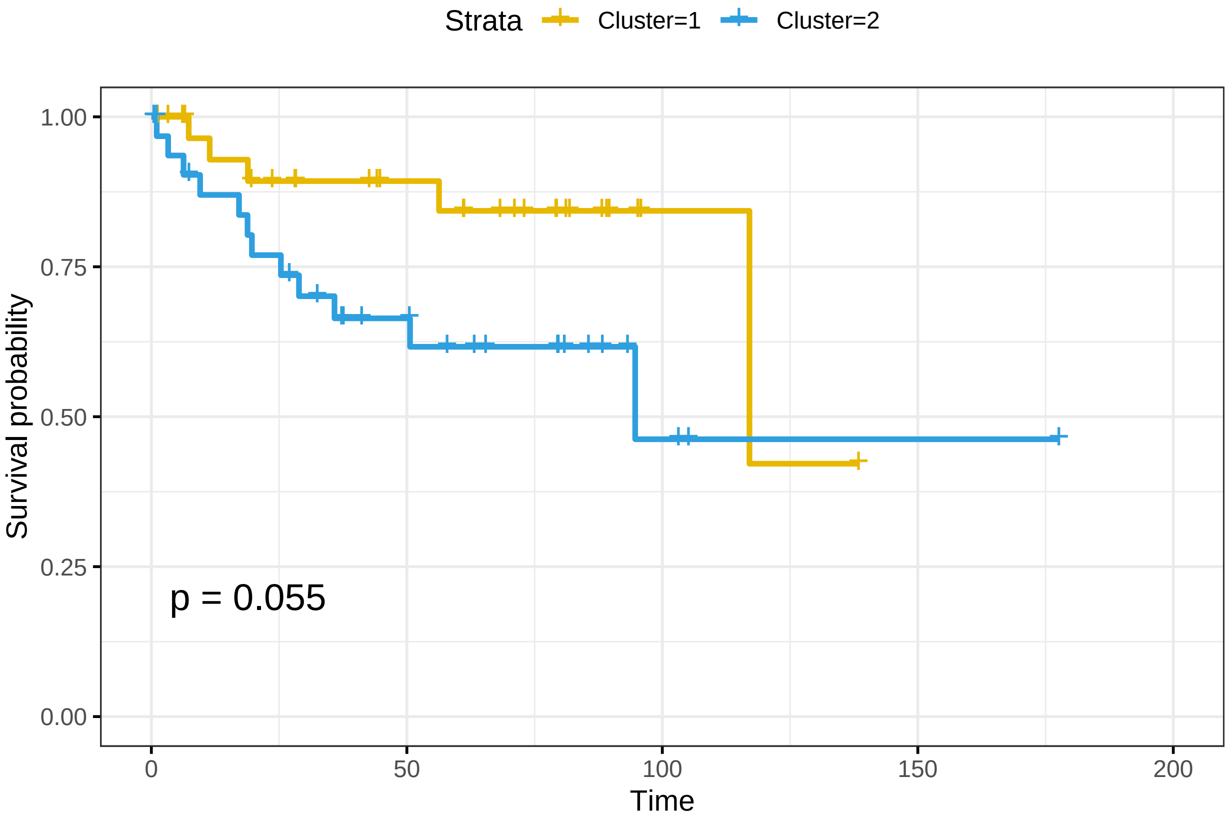


Figure 18: Kaplan-Meier plot with clusters from figure 4 (script 5) (n = 82)

d- Non selected significant coxph CTA genes clustering

```
# Prepare table
l <- read.table("../results/clusters_indiv/clusters_indiv_signif_non_selected_cta_all.tsv",
    sep = "\t", header = TRUE)

df_cluster_all <- merge(l, df_metadata_surv_conv, by = "Patient")
rownames(df_cluster_all) <- df_cluster_all$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_all),
    df_cluster_all)
p
```

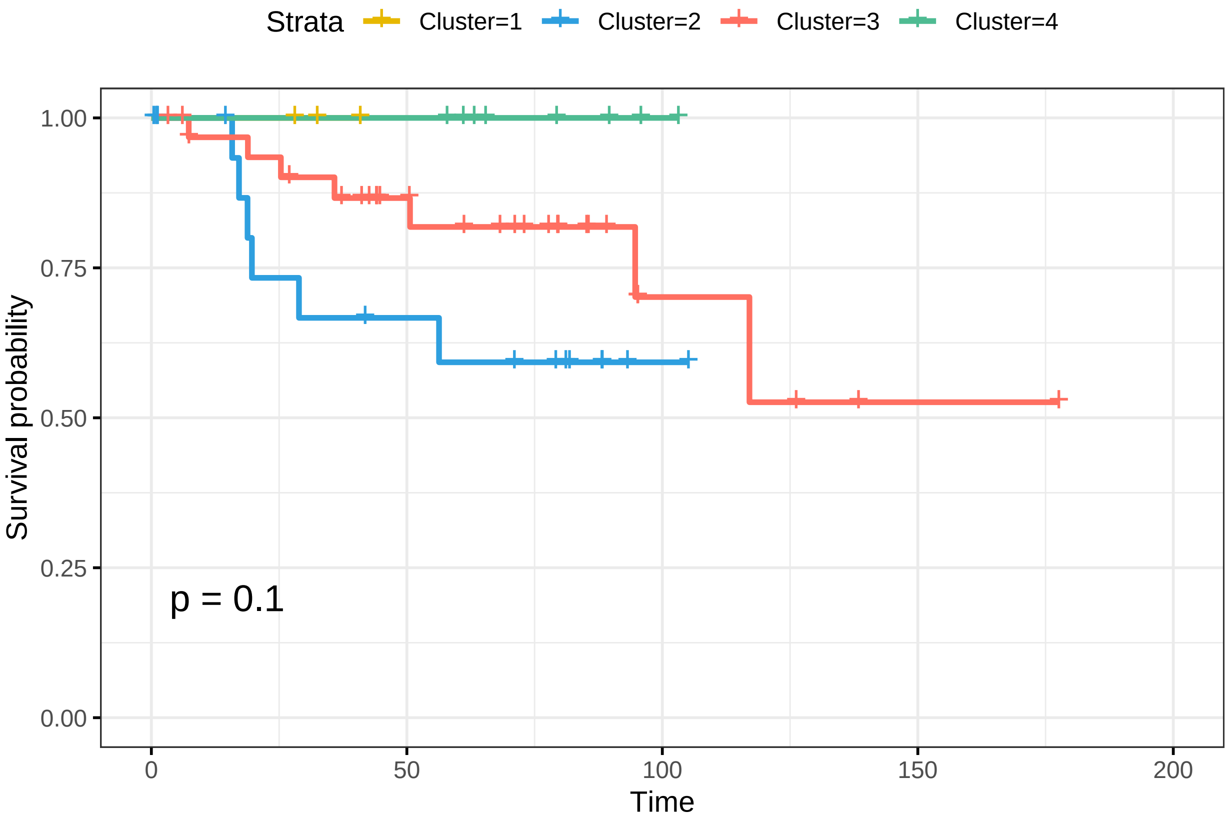


Figure 19: Kaplan-Meier plot with clusters from figure 5 (script 5) (n = 82)

```
# Prepare table
df_cluster_conv_merge <- merge(l, df_metadata_surv_conv, by = "Patient")
df_cluster_conv_merge$Cluster <- ifelse(df_cluster_conv_merge$Cluster ==
    2, 1, df_cluster_conv_merge$Cluster)
df_cluster_conv_merge$Cluster <- ifelse(df_cluster_conv_merge$Cluster ==
    4, 3, df_cluster_conv_merge$Cluster)
df_cluster_conv_merge$Cluster <- ifelse(df_cluster_conv_merge$Cluster ==
    3, 2, df_cluster_conv_merge$Cluster)
rownames(df_cluster_conv_merge) <- df_cluster_conv_merge$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_conv_merge),
    df_cluster_conv_merge)
p
```

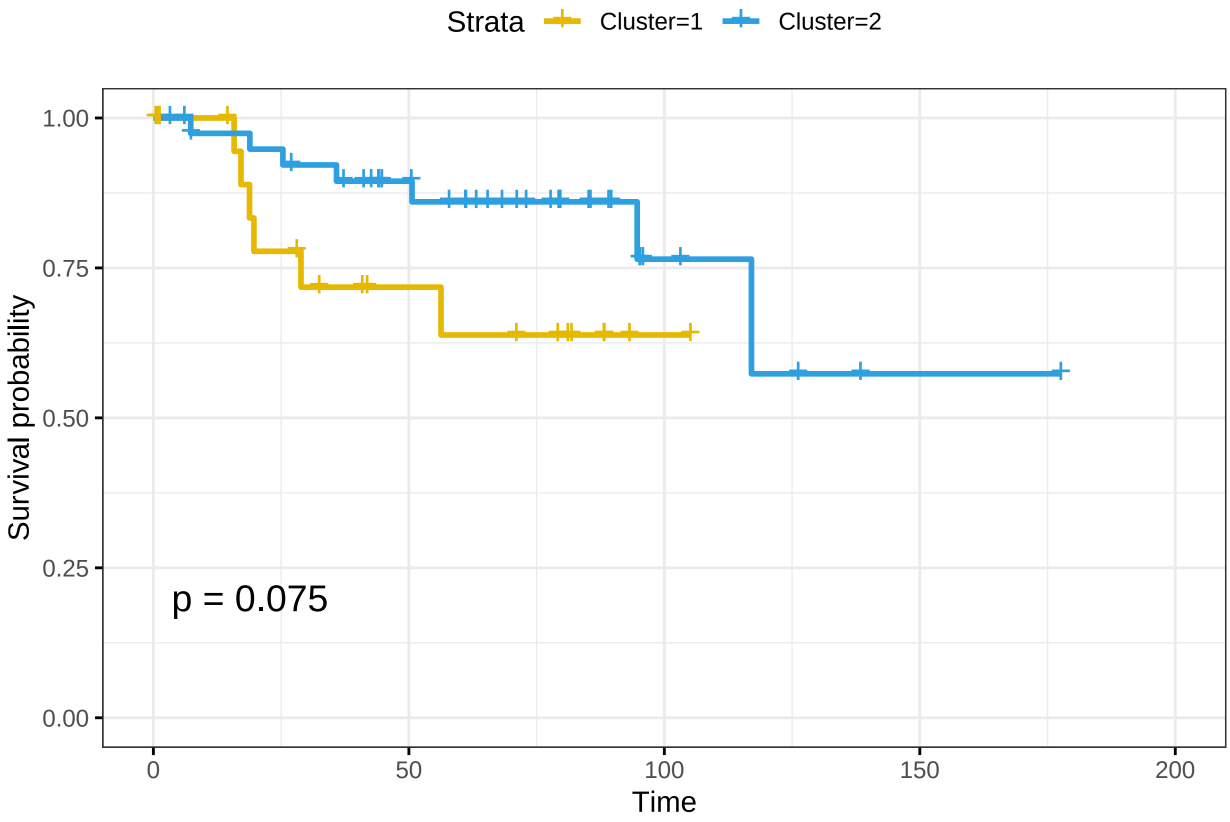


Figure 20: Kaplan-Meier plot merged clusters from figure 5 (script 5) (n = 82)

Merge C1 with C2 and C3 with C4

2) Conventional chondrosarcomas

This part concerns conventional types.

a- Clustering Kmeans k = 2

This clustering is from figure 33.

```r
# Read clusters
l_anno_conv <- read.table("../results/clusters_indiv/clusters_conv_indiv_mhc.tsv",
    header = TRUE, sep = "\t")
df_cluster_conv <- merge(l_anno_conv, df_metadata_surv_conv,
    by = "Patient")
rownames(df_cluster_conv) <- df_cluster_conv$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_conv),
    df_cluster_conv)
p
```

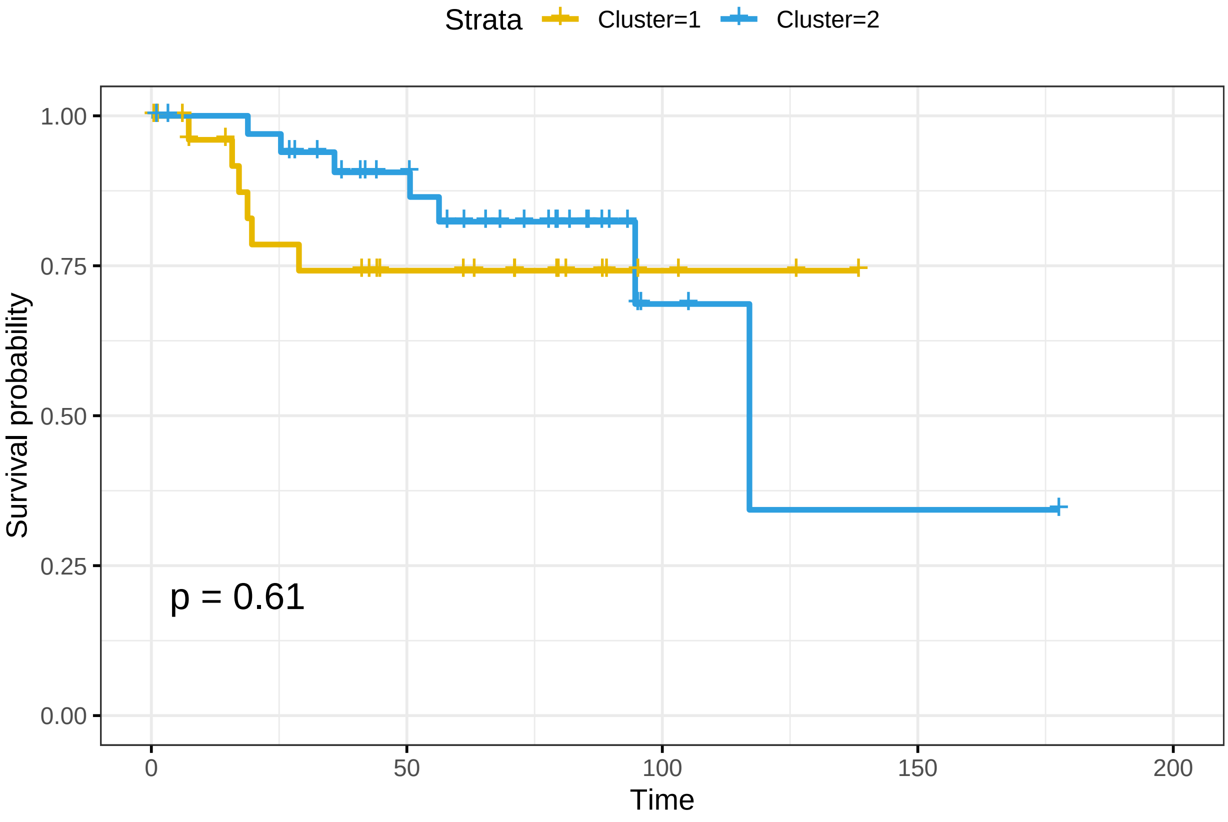


Figure 21: Kaplan-Meier plot with clusters from figure 31 (script 5) (n = 63)

We see that there is not differences.

b- Significative coxph CTA genes clustering

This clustering is from heatmap 3.

```r
# Read clusters
l_cta_conv <- read.table("../results/clusters_indiv/clusters_conv_indiv_mhc_cta_signif_coxph.tsv",
    header = TRUE, sep = "\t")

# Prepare table
df_cluster_cta_all <- merge(l_cta_conv, df_metadata_surv, by = "Patient")
rownames(df_cluster_cta_all) <- df_cluster_cta_all$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_cta_all),
    df_cluster_cta_all)
p
```

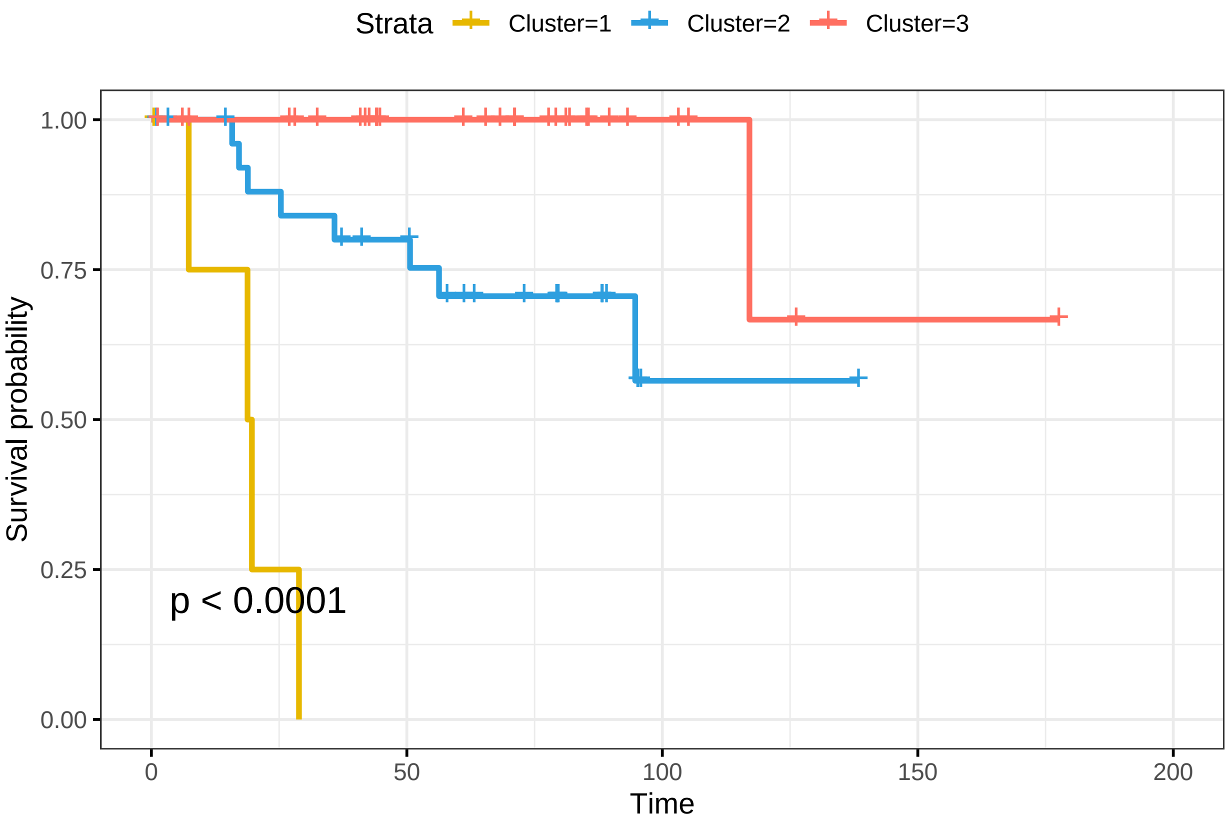


Figure 22: Kaplan-Meier plot with clusters from figure 6 (script 5) (n = 63)

This confirm the KM plot with all patients. We clearly see that some CTA have bad impact on survival.

Merge C1 and C2 Here, we want to merge the 2 clusters to see the differences than with 3.

```
# Prepare table
df_cluster_cta_all_merge <- merge(l_cta_conv, df_metadata_surv,
    by = "Patient")
df_cluster_cta_all_merge$Cluster <- ifelse(df_cluster_cta_all_merge$Cluster ==
    2, 1, df_cluster_cta_all_merge$Cluster)
rownames(df_cluster_cta_all_merge) <- df_cluster_cta_all_merge$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_cta_all_merge),
    df_cluster_cta_all_merge)
p
```

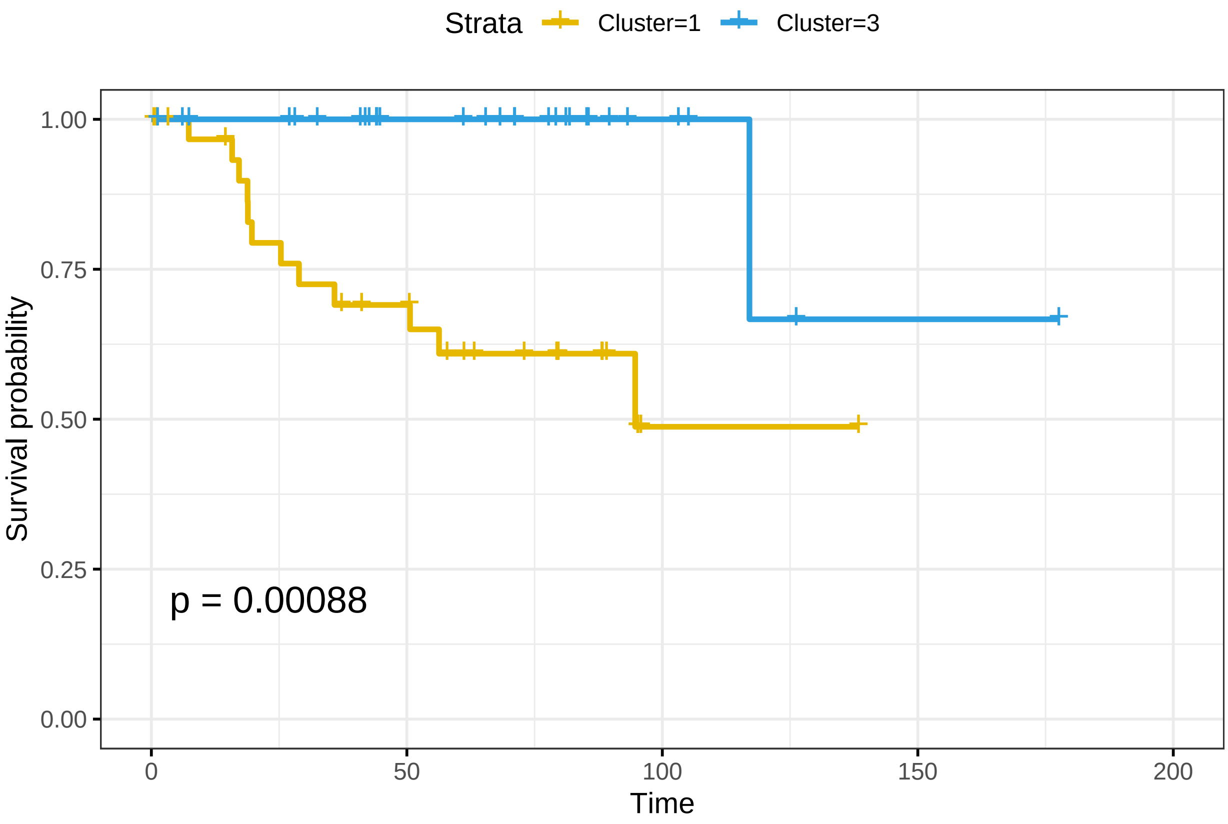


Figure 23: Kaplan-Meier plot with merged clusters from figure 6 (n = 63)

We see that there is always significant.

c- Selected significant coxph CTA genes clustering

```
# Prepare table
l <- read.table("../results/clusters_indiv/clusters_indiv_signif_selected_cta_conv.tsv",
    sep = "\t", header = TRUE)

df_cluster_conv <- merge(l, df_metadata_surv_conv, by = "Patient")
rownames(df_cluster_conv) <- df_cluster_conv$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_conv),
    df_cluster_conv)
p
```

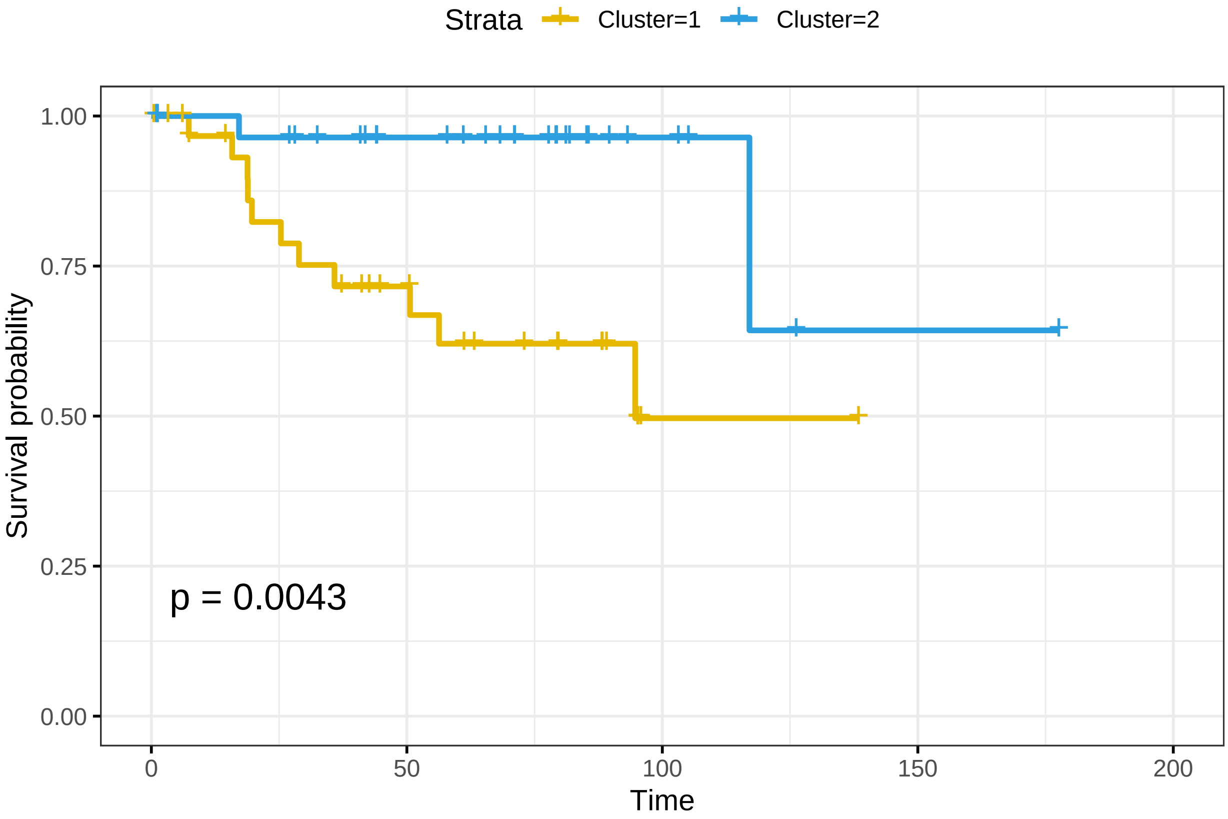


Figure 24: Kaplan-Meier plot with clusters from figure 7 (script 5) (n = 63)

d- Non selected significant coxph CTA genes clustering

```
# Prepare table
l <- read.table("../results/clusters_indiv/clusters_indiv_signif_non_selected_cta_conv.tsv",
    sep = "\t", header = TRUE)

df_cluster_conv <- merge(l, df_metadata_surv_conv, by = "Patient")
rownames(df_cluster_conv) <- df_cluster_conv$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_conv),
    df_cluster_conv)
p
```

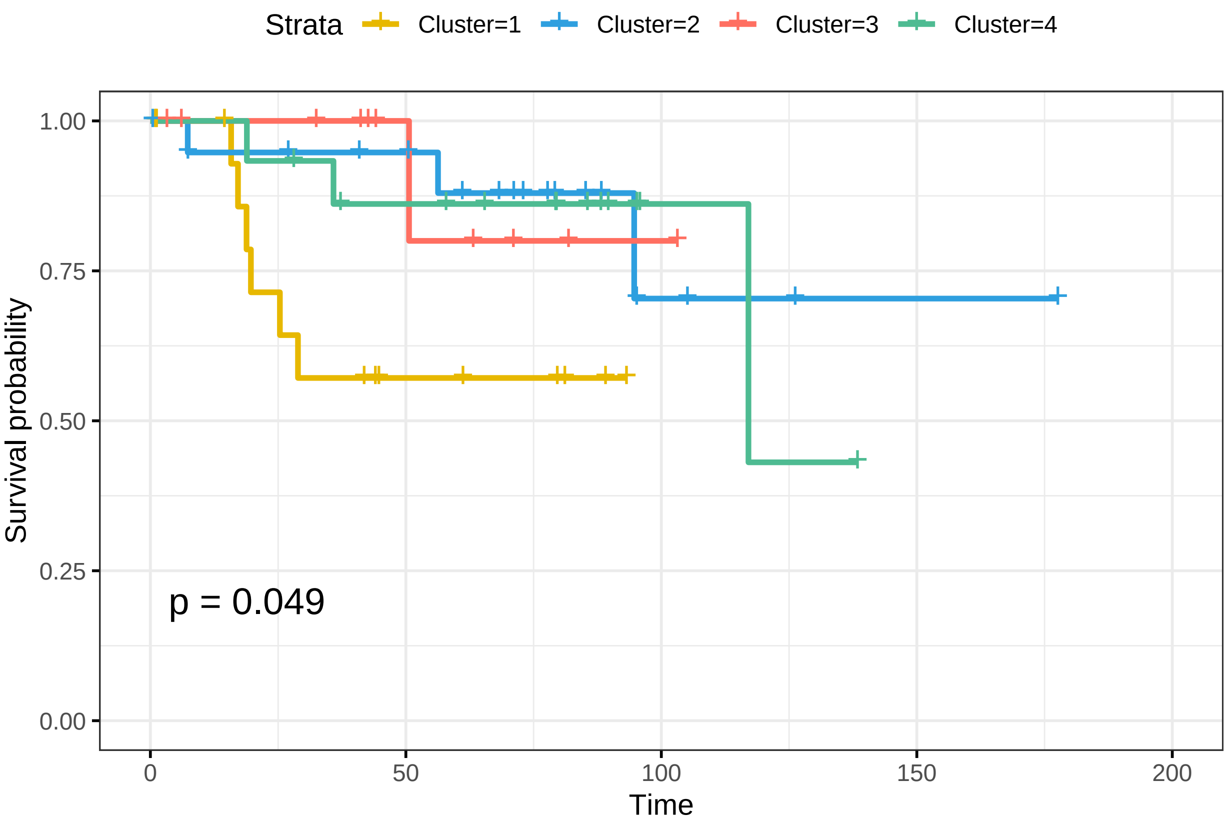


Figure 25: Kaplan-Meier plot with clusters from figure 8 (script 5) (n = 63)

```r
# Prepare table
df_cluster_conv_merge <- merge(l, df_metadata_surv_conv, by = "Patient")
df_cluster_conv_merge$Cluster <- ifelse(df_cluster_conv_merge$Cluster ==
    2, 1, df_cluster_conv_merge$Cluster)
df_cluster_conv_merge$Cluster <- ifelse(df_cluster_conv_merge$Cluster ==
    4, 3, df_cluster_conv_merge$Cluster)
df_cluster_conv_merge$Cluster <- ifelse(df_cluster_conv_merge$Cluster ==
    3, 2, df_cluster_conv_merge$Cluster)
rownames(df_cluster_conv_merge) <- df_cluster_conv_merge$Patient
p <- plot_km(survfit(Surv(OS.delay, OS.event) ~ Cluster, data = df_cluster_conv_merge),
    df_cluster_conv_merge)
p
```

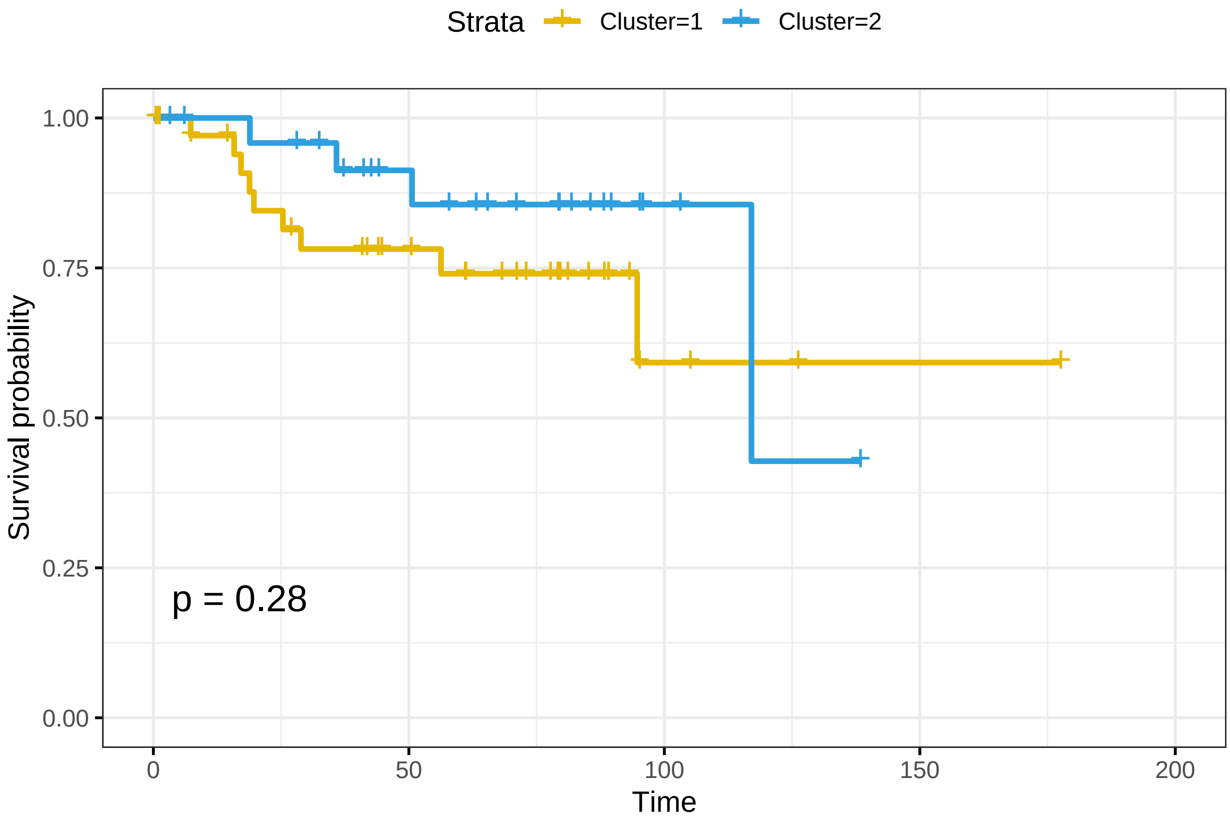


Figure 26: Kaplan-Meier plot merged clusters from figure 8 (script 5) (n = 63)

Merge C1 with C2 and C3 with C4

3) Cluster 1 from CTA heatmaps analysis

This section is the analysis of the previous results. We want to see where the patients clustered in other heatmaps.

```
# Take indiv from cluster 1
l_c1_cta_all <- l_cta_all[l_cta_all$Cluster == 1, ]

# Merge to see where the indiv are in the other heatmap
df_analysis_cold_all <- l_c1_cta_all %>%
    left_join(df_metadata %>%
        select(Patient, Histology), by = "Patient") %>%
    left_join(l_anno_all, by = "Patient")
df_analysis_cold_all
```

```
##    Cluster.x Patient        Histology Cluster.y
## 1          1   CN027 dedifferentiated        NA
## 2          1   TR031 dedifferentiated        NA
## 3          1   LY040 dedifferentiated        NA
## 4          1   CN012 dedifferentiated        NA
## 5          1   TR062 dedifferentiated        NA
## 6          1   TE063 dedifferentiated        NA
## 7          1   Li010 dedifferentiated        NA
## 8          1   LY016               G3         1
## 9          1   TE015               G3         1
## 10         1   TR016 dedifferentiated        NA
## 11         1   TR022               G3         1
## 12         1   CN019               G3         1
## 13         1   TE017               G3         1
## 14         1   LY019               G2         1
## 15         1   CN033               G3         1
## 16         1   CN008               G3         1
## 17         1   TR042 dedifferentiated        NA
## 18         1   TR004 dedifferentiated        NA
```

We see that there is a lot of dediff type that expressed CTA involved in bad survival events but retrieve in cluster 4 (HOT) for the immune cells. One dediff is in the cold cluster. So the differentiated cancers are really different than conventional and are infiltrated by immune cells or not but the most is infiltrated.

```
# Take indiv from cluster 1
l_c1_cta_conv <- l_cta_conv[l_cta_conv$Cluster == 1, ]
df_analysis_cold_conv <- l_c1_cta_conv %>%
    left_join(df_metadata %>%
        select(Patient, Histology), by = "Patient") %>%
    left_join(l_anno_all, by = "Patient") %>%
    left_join(l_anno_conv, by = "Patient")
df_analysis_cold_conv
```

```
##   Cluster.x Patient Histology Cluster.y Cluster
## 1         1   LY016        G3         1       1
## 2         1   TE015        G3         1       1
## 3         1   CN033        G3         1       1
## 4         1   CN019        G3         1       1
## 5         1   CN008        G3         1       1
```

For the conventional chondrosarcomas, we see that all the patient have G3 chondrosarcoma and can be in the cluster 2.