1 Results

Some more guidelines from the School of Geosciences.

This section should summarize the findings of the research referring to all figures, tables and statistical results (some of which may be placed in appendices).

- include the primary results, ordered logically - it is often useful to follow the same order as presented in the methods.
- alternatively, you may find that ordering the results from the most important to the least important works better for your project.
- data should only be presented in the main text once, either in tables or figures; if presented in figures, data can be tabulated in appendices and referred to at the appropriate point in the main text.

Often, it is recommended that you write the results section first, so that you can write the methods that are appropriate to describe the results presented. Then you can write the discussion next, then the introduction which includes the relevant literature for the scientific story that you are telling and finally the conclusions and abstract – this approach is called writing backwards.

1.1 Determination of inflammatory groups representing intratumor inflammatory status

Functional clusters of osteosarcoma samples were created using k-means clustering of from MDS visualization of the Hallmark Inflammatory Response signature.

```
root_dir <- "../TargetOS-Osteosarcoma/"
knitr::opts_knit$set(root.dir = root_dir)
```

Inflammatory groups characterizing the intensity of inflammatory status in tumors were created by first creating groups of inflammatory status. The number of groups were tried using k-means clustering algorithm on a MDS visualization of the scaled by Z-score of the 88 tumor samples for the hallmark inflammatory response signature from MSigDB, containing 200 genes. ICAM4 was notably not detected in the dataset in the TARGET-OS cohort. Thus, 199 genes from the signature were used.

The groups are chosen from Figure 1.C as they are relevant as they correspond fairly well to functional groups, defined by k-means clustering based on MDS visualization **(Figure ??)**. Each sample is thus attributed to its inflammatory status and this group will be subsequently used for the following results.

1.2 Characterization of osteosarcomas associated to inflammatory status

Comparison of the mean of *Hp Osteosarcoma* gene signature and gene relating to types of osteosarcomas associated to inflammatory groups were performed, represented through a heatmap. Despite high heterogeneity between samples and inflammatory status, the Z-score of the mean of genes in Low versus High group is statistically significatively different.

However *Hp Osteosarcoma* in GSEA is not significantly differrent ($p = 0.163$).

Comparison of the expression of specific osteosarcoma markers relating to osteoblastic, chondroblastic, fibroblastic markers through a heatmap representation. Hierarchical clustering of the samples does not appear to be associated with corresponding inflammatory status. However it does reveal that there are groups of osteoblastic, chondroblastic and fibroblastic osteosarcomas which is expected.

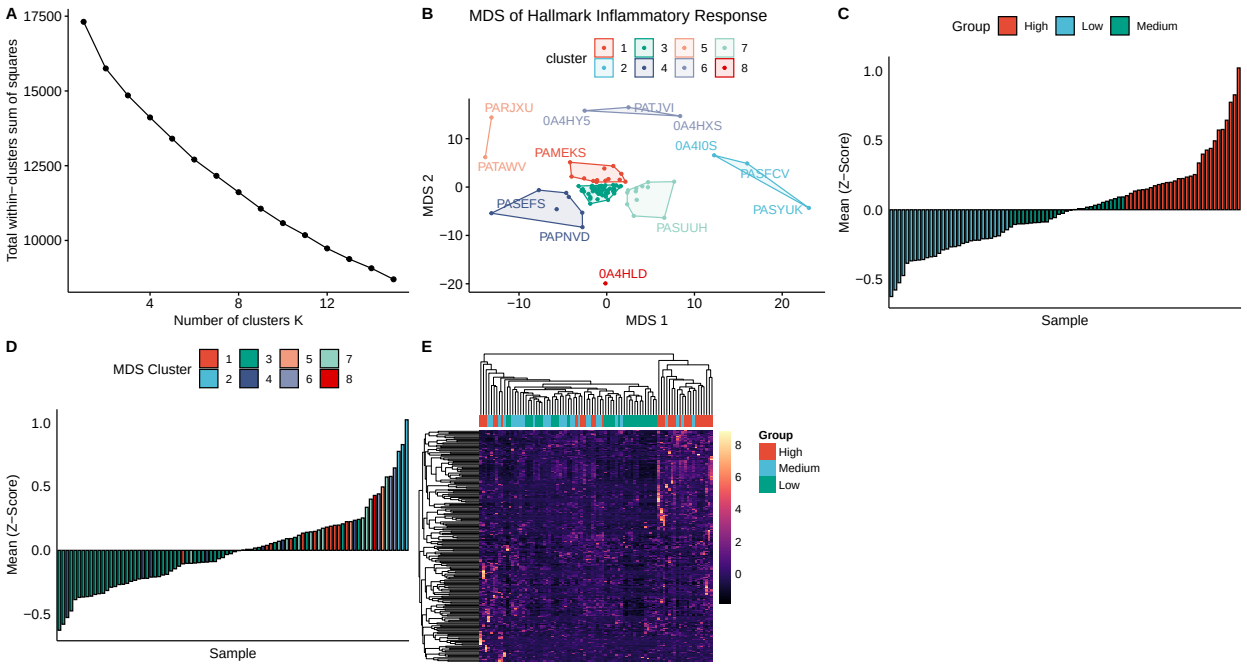

Figure 1: Construction of inflammatory groups

The mean of markers of proliferation (MKI67, PCNA, TOP2A) associated to osteosarcomas have been compared to inflammatory status, along with the mean of the three markers. Kruskal-Wallis testing is significant ($p = 0.00968$) and post-hoc Dunn analysis reveals that the mean of the proliferation markers between low and high group is significantly different ($p = 0.016$). The data suggests that proliferation is hindered when inflammatory status is high in the osteosarcoma samples.

```
hm_ost %<>% as.ggplot()
hm_ost_c %<>% as.ggplot()
hm_os_type %<>% as.ggplot()
hm_os_type_mean %<>% as.ggplot()
pho <- ggarrange(hm_ost, hm_ost_c, hm_os_type, hm_os_type_mean, labels = "AUTO")

suppressWarnings(print(pho))
```

1.3 Characterization of intra-tumor inflammation associated to inflammatory status

1.3.1 Survival curve

```
suppressWarnings(print(ggsurv))
```

1.4 Similarity of inflammatory signatures

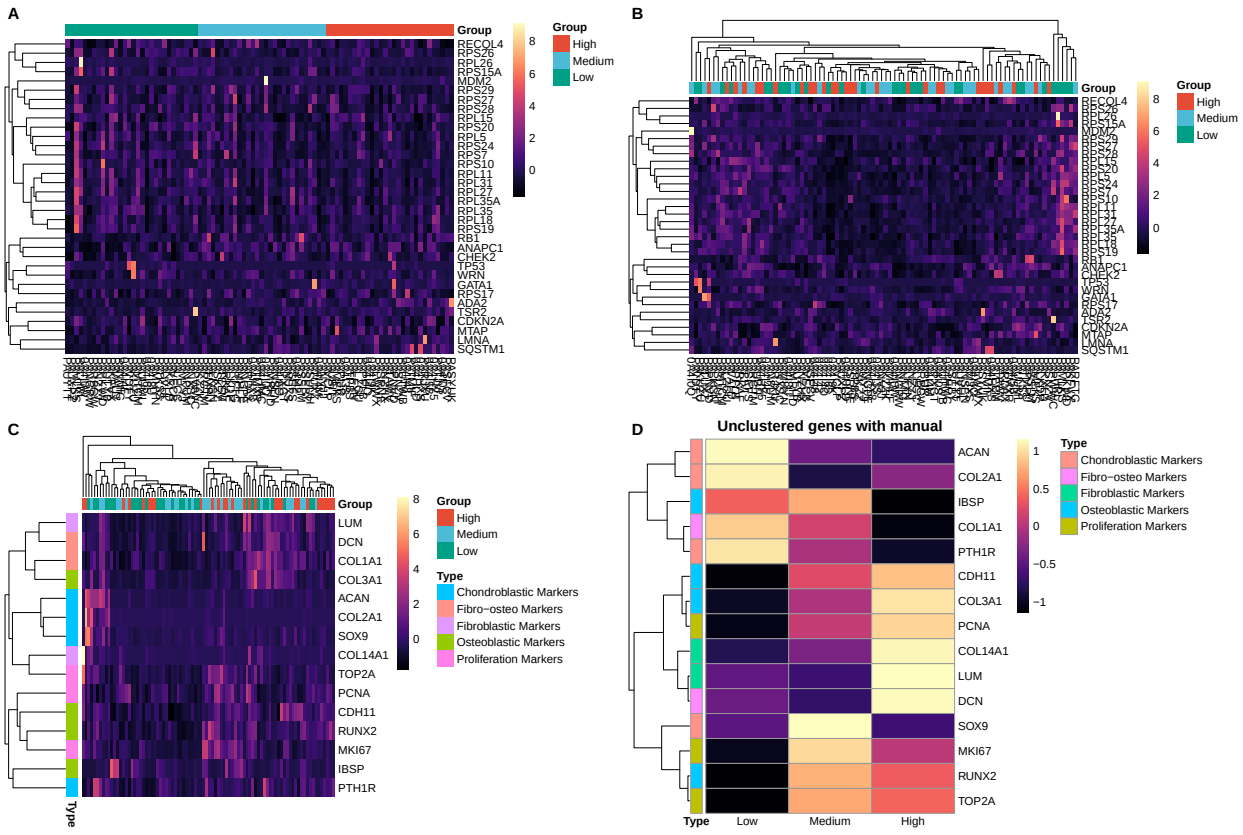

Figure 2: (#fig:osteosarcoma_plot)**Heatmap of HP Osteosarcoma signature with samples annotated to their respective inflammatory groups.**

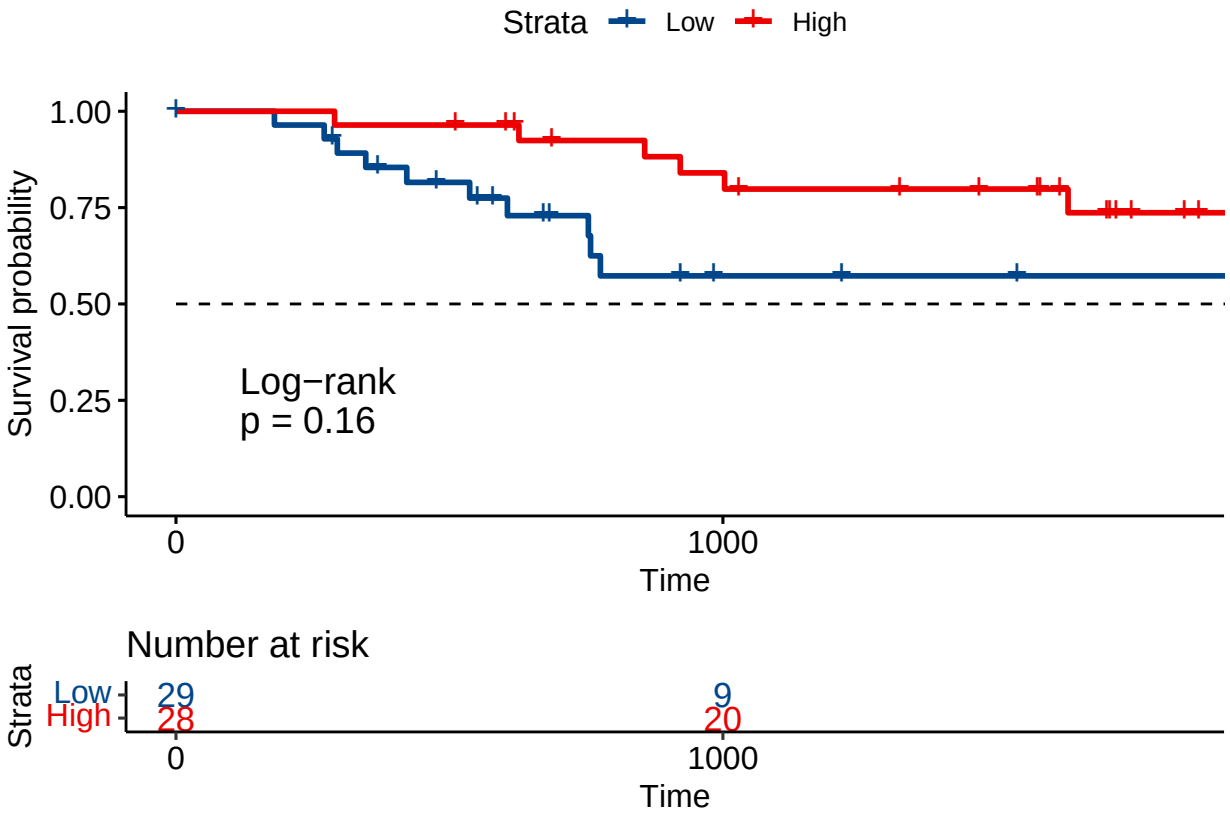

Figure 3: **Kaplan-Meier survival plot of low vs high group indicate a trend associating inflammatory status with survival prognostic.** Horizontal and vertical axes represent survival times and rates, respectively. Red and blue curves are samples with risk score higher and lower than the median value, respectively. Plus signs indicate censored values. Depicted P-values were obtained by the logrank test.

```
knitr::include_graphics("../04_figures/ggplot/Venn_estimate_vs_hallmark_inflammatory_response.png")
```

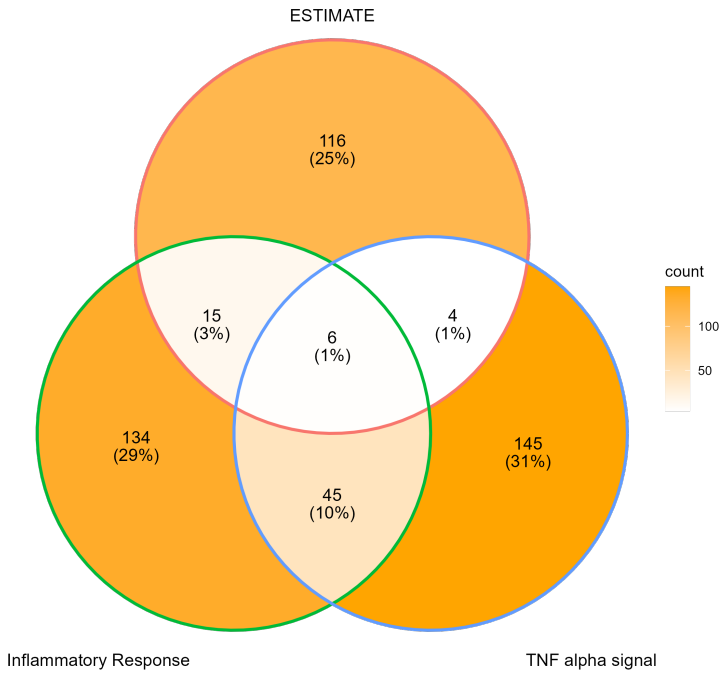

Figure 4: Venn diagram comparing three inflammatory signatures.

1.5 Relationship of inflammatory status with immune response

1.6 Immune abundance by immune deconvolution algorithm

Immune cell abundance can be determined thanks to immune deconvolution algorithm. Here, MCP-Counter and CIBERSORT

1.7 single cell RNA-Seq analysis

Using scRNA-Seq data from GSE152048, used by @Zhou.202005 and normalized by Sophie