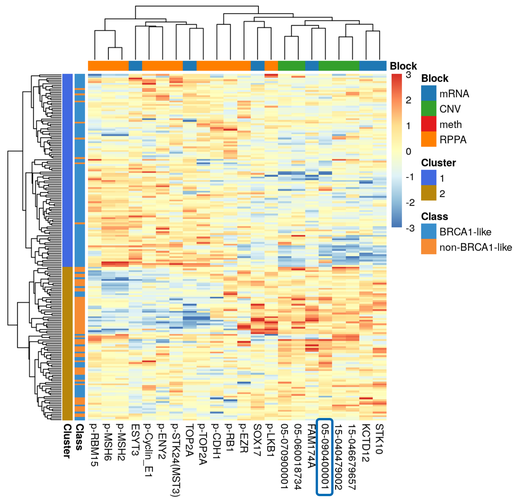
[FPN: 119P] Characterising Homologous Recombination Deficient Triple-negative Breast Cancers with a Multiomics Approach

1. Background

- **Homologous recombination deficiency (HRD):**
  - Inability to repair DNA double strand breaks in an error-free manner
  - Common in triple-negative breast cancers
  - Detectable via patterns of DNA copy number aberrations → **BRCA1-like phenotype**
  - Patients benefit from (intensified) **platinum-based chemotherapies (IPB-chemo)**,
    - *but not always* → *which patients and why?*

2. BRCA1-like multiomics features

Sparse generalised canonical correlation analysis (sGCCA, see Methods) identified 22 features:



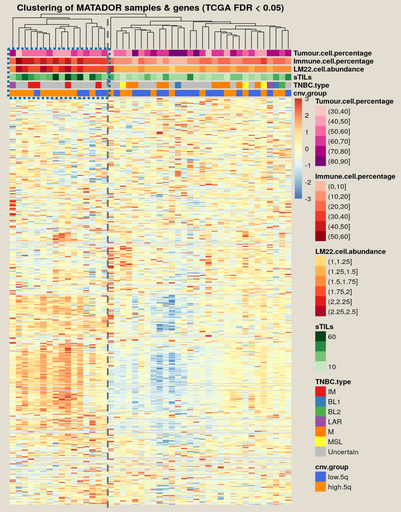
- Unsupervised clustering → *evidence of subgroups* among BRCA1-like (HRD) tumours

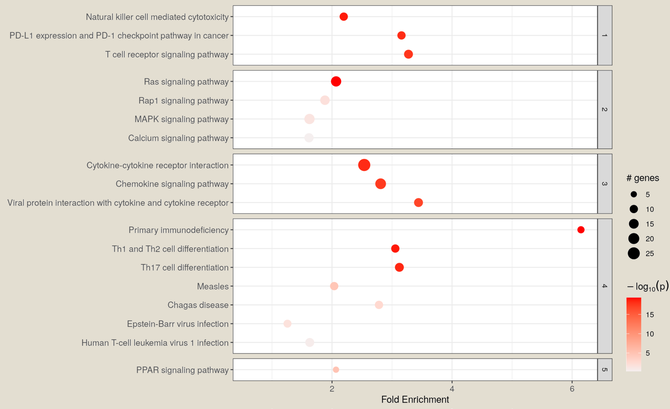
CNV 5q14.3: platinum sensitivity marker?

- Copy-number variation (CNV) sequencing read-out at 5q14.3
  - Defining feature of the clusters ($p = 0.01$)
  - Copy number *loss* is associated with *worse* survival outcome after IPB-chemo ($p < 0.001$)

3. DGE and pathway enrichment analysis

Result of differential gene expression (DGE) analysis (factor: CNV 5q14.3 read-out)

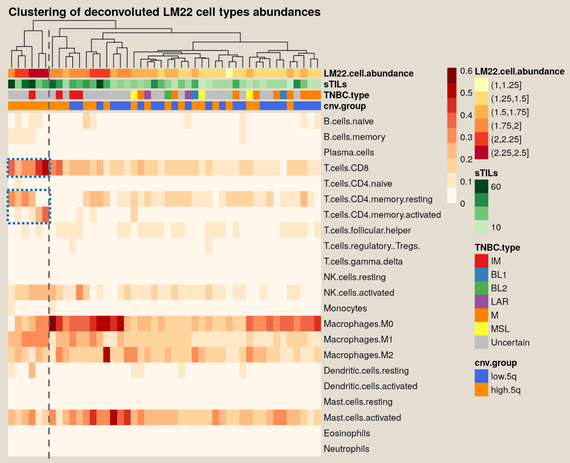




- 525 differentially expressed genes ($\mathrm{FDR} < 0.05$)
- Validated in separate data set (TCGA→MATADOR)
- Associated with *sTILs* and *immune cells abundance (LM22 absolute score)*
- Enriched pathways are *immune system related*, e.g.:
  - PD-L1 expression and PD-1 checkpoint ($p = 6.41 \times 10^{-19}$)
  - Natural killer cell mediated cytotoxicity ($p = 1.42 \times 10^{-19}$)
  - Primary immunodeficiency ($p < 10^{-20}$)

4. Immune cell type deconvolution

Result of immune cell type deconvolution analysis (CIBERSORTx, see Methods)



- The subgroup *without 5q14.3 copy number loss* (~ *higher sTILs*, and *better survival outcome after IPB-chemo*) is also associated with
  - higher CD8+ T cells fraction ($p = 0.02$), and
  - higher CD4+ memory T cells fraction ($p = 0.01$),

  in bulk tumour samples.

5. Conclusions and Discussion

- Evidence for a subgroup of BRCA1-like (HRD) TNBC patients that:
  - do *not* exhibit a *5q14.3 copy number loss*,
  - possess *immunomodulatory features*,
  - are associated with *higher sTILs and immune cells abundance*, and
  - are *sensitive to platinum-based chemotherapies*†
- We note that CNV read-outs can be convoluted by tumour cell percentages in bulk samples
- A more detailed cell type deconvolution might provide more insights
- † The predictive values of the identified features are still to be validated

Methods

Sparse Generalised Canonical Correlation Analysis (sGCCA): BRCA1-like status of TCGA and RATHER triple-negative breast cancer (TNBC) samples (*n* = 84 and 112) were identified using a pretrained shrunken nearest centroid CNV classifier. sGCCA was performed using the `mixOmics` R package to identify CNV, gene expression, protein expression (RPPA) and DNA methylation features most associated with BRCA1-like status.

Identifying platinum sensitivity marker: Unsupervised hierarchical clustering was performed on the BRCA1-like multiomics features identified by sGCCA, using TCGA and RATHER TNBC samples. Logistic regression of the top-level clusters on the multiomics features was performed. Logistic regression of platinum sensitive/non-sensitive N4+ patient groups on the most significant multiomics feature (CNV 5q14.3) was subsequently performed.

DGE and pathway enrichment analysis: BRCA1-like TNBC samples from TCGA and MATADOR (*n* = 54) were separated into two groups by their CNV 5q14.3 read-outs, split at the median value. DGE analysis was performed on the mRNA-seq data of the two groups using the `edgeR` R package. Pathway enrichment analysis via active subnetwork search was performed using the `pathfindR` R package with the KEGG pathway database and a greedy search algorithm.

Immune cell type deconvolution: Cell type deconvolution for the TCGA and MATADOR bulk tumour samples was performed using the `CIBERSORTx` algorithm and the LM22 signature matrix.

Breast cancer cohorts:

TCGA: www.cancer.gov/tcga
RATHER : ratherproject.com
N4+: NCT03087409
MATADOR: ISRCTN61893718
(Data availability statement)

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