UNIVERSITÀ di VERONA

Dipartimento di SCIENZE CHIRURGICHE ODONTOSTOMATOLOGICHE E MATERNO-INFANTILI

RELAZIONE SCIENTIFICA ANNUALE

Assegno di Ricerca (AdR 3455/20)

Nome e Cognome del Beneficiario	VALENTINA MENGARDO
Titolo del Programma di Ricerca	IDENTIFICAZIONE DEI PATHWAY MOLECOLARI DI PROGRESSIONE E DI STRATEGIE DI TRATTAMENTO TAILORED NEI TUMORI DELLO STOMACO CON ISTOTIPO POORLY COHESIVE E SIGNET RING CELLS
Settore Scientifico Disciplinare di riferimento	MED/18 Chirurgia generale
Nome e Cognome del Responsabile Scientifico	prof. Giovanni de Manzoni
Durata dell'Assegno di Ricerca (da…a…)	Da 01/03/2020 a 28/02/2021
Periodo di riferimento della relazione (da…a…)	Da 01/03/2020 a 28/02/2021
Note (es.: eventuali periodi di sospensione dell'Assegno, etc.)	

RISULTATI DELLA RICERCA

During the last year, I was actively involved in research activity focused on Poorly Cohesive (PC) gastric cancer (GC) under the supervision of Prof. Giovanni de Manzoni and Dr. Maria Bencivenga. In detail, on behalf of the European Chapter of International Gastric Cancer Association, including some of the most relevant European surgeons, pathologists and oncologists in the field of GC, we first proposed a new classification of PC type based on the amount of SRCs.

Specifically, PC tumors were coded into three categories:

1) "pure" Signet Ring Cell cancers having ≥90% of signet ring cells (SRCC);

2) Poorly Cohesive carcinoma with signet ring cell component between >10% and <90% (PCC/SRC);
3) PC not otherwise specified carcinoma with ≤10% of signet ring cells (PCC-NOS).

Then, by using such classification, we performed a multicenter European study. We found that the proportion of SRCs was inversely related to the depth of tumor invasion defined by pathological tumor and nodal status. In multivariable analysis, the amount of SRCs was shown to have an independent impact on cancer-related survival, with "pure" SRCC tumors having the best prognosis among PC categories.

Based on this, we further hypothesized that the comparison of differential genomic and proteomic profiles between groups of PC cancers with different tumor stage (early vs advanced) and different morphology ("pure" SRCC vs PCC/SRC and PCC-NOS) could enable to identify the molecular mechanisms of tumor progression in this GC subtype. This would hopefully pave the way to identify tailored treatment options, allow a proper selection of patients in clinical trials and ultimately improve prognosis.

To this purpose, we provided some preliminary results by analyzing PC tumors treated at Upper GI Surgery of Verona University that were part of the clinical multicenter study reported above. We evaluated, the correlation between tumor stage/morphology and genomic/gene expression profiles as described in detail below:

1. 64 PC cases were analyzed for 409 genes included in the TML assay panel (Thermofisher). We observed that tumor mutational load (TML) tended to increase when the number of signet ring cells decreases, that is in "undifferentiated" PC tumors (P <0.0001). TML over 10 mutations/Mb was significant associated with patients' worst survival (p = 0.0043).

   Mutations were found in at least one gene in 49 of the 64 cases. The most frequent mutations involved CDH1 (21/64; 32.8%), followed by TP53 (20/64; 31.3%) and PIK3CA (7/64; 10.9%). Of note, FGFR2, FGFR1, IGFR1 mutations which are typical of Epithelial to Mesenchymal Transition (EMT), were found only in "less differentiated" PCC/SRC and PCC-NOS cases.

2. Gene expression profile was analyzed in 30 PC cases. We identified 1936 high variable genes (**Figure 1A**). Principal component analysis (PCA) and dendrogram, depicted in **Figure 1B** and **Figure 1C** respectively, identified 4 gene clusters (**Figure 2**). Differential expression (DE) analysis identified 367 genes differentially expressed among the 4 clusters. Among the differently expressed genes, we found some that identified each cluster. In order to better characterize the 4 clusters, we performed Gene Set Enrichment Analysis (GSEA) on each cluster by comparing it with the sum of the other three remaining ones. In particular, we selected only the 367 differentially expressed genes to better highlight cluster differences. Using only the "hallmark gene sets" present in the "MSigDB Collections" database (www.gsea-msigdb.org/gsea/msigdb/collections.jsp), we identified that cluster A differed from the others for "epithelial to mesenchymal transition" (EMT) and "myogenesis" signatures while cluster B was enriched as well as EMT also for "G2M checkpoint", "E2F targets" and "mitotic spindle" signatures. Otherwise, cluster C and D showed no enrichment in any of the "Hallmark" signatures (**Figure 3A and**

3B). Furthermore, we calculated for each sample its gene set variation analysis (GSVA) score for "Hallmark" pathways. In order to compare the impact of each pathway in each cluster, the median of the GSVA scores was calculated and a significant p-value according to Benjamini and Hochberg was considered. We observed that the GSVA score for the EMT pathway descended from cluster A to cluster D while the "E2F targets", "G2M checkpoint" and "MYC targets" pathways were highly specific to cluster B (**Figure 3C**). Finally, analyzing mutated genes for each cluster, we observed that cluster A included 3 cases affected by a mutation in the *BAP1* gene (p-value = 0.012) while cluster B presented 4 cases with mutations in the *PIK3CA* gene (p-value = 0.0497). We then compared the overall survival between grouped samples based on gene expression analysis and observed that that the samples of cluster D had the best prognosis unlike those of cluster A who had the worst prognosis (**Figure 4A**). The comparison is statistically significant when the samples of cluster A and B (those with EMT signatures) are compared with those of clusters C and D (p-value = 0.0095; **Figure 4B**).

As showed in **Figure 5,** the activation of EMT pathway is associate to increasing biological aggressiveness of PC carcinoma. Unfortunately, due to the small sample size (n=30) available for expression analysis we couldn't find significant association between tumor stage nor with percentage of SRCs in the tumor, even if no early (stage I) or even stage II GC were found in clusters A and B, moreover SRC percentage tends to be higher in the cluster D.

Our preliminary data suggest that indeed the acquisition of molecular alterations of EMT could be the mechanism of progression of aggressive advanced PC tumors.

We are now going on with the research on this topic, moreover I am actively involved in the prospective collection of clinical data and tissue samples from patients who are referred to the Unit of Upper GI Surgery of Verona University due to newly diagnosed Poorly Cohesive gastric cancer.

Please note that Figures are available in the attached file.

DESCRIZIONE DELL'ATTIVITÀ SCIENTIFICA/DIDATTICA COLLEGATA

The present research grant has allowed the beneficiary to collaborate in other projects, by research affinity, aimed at improving the treatment of patients with gastric and esophageal cancer.
This collaboration led to the publication of 10 articles in international scientific journals and another article is currently "under review". Furthermore, this grant allowed the beneficiary to be part of the Steering Committee of an Italian randomized multicentre Trial of which the University of Verona is neither the promoter (acronym: ADiGe Trial) in collaboration with the GIRCG association.
In particular:

- Weindelmayer J, **Mengardo V**, Veltri A, Torroni L, Zhao E, Verlato G, de Manzoni G. Should we still use prophylactic drain in gastrectomy for cancer? A systematic review and meta-analysis. Eur J Surg Oncol. 2020 Aug;46(8):1396-1403. doi: 10.1016/j.ejso.2020.05.009. Epub 2020 May 15. PMID: 32457016.

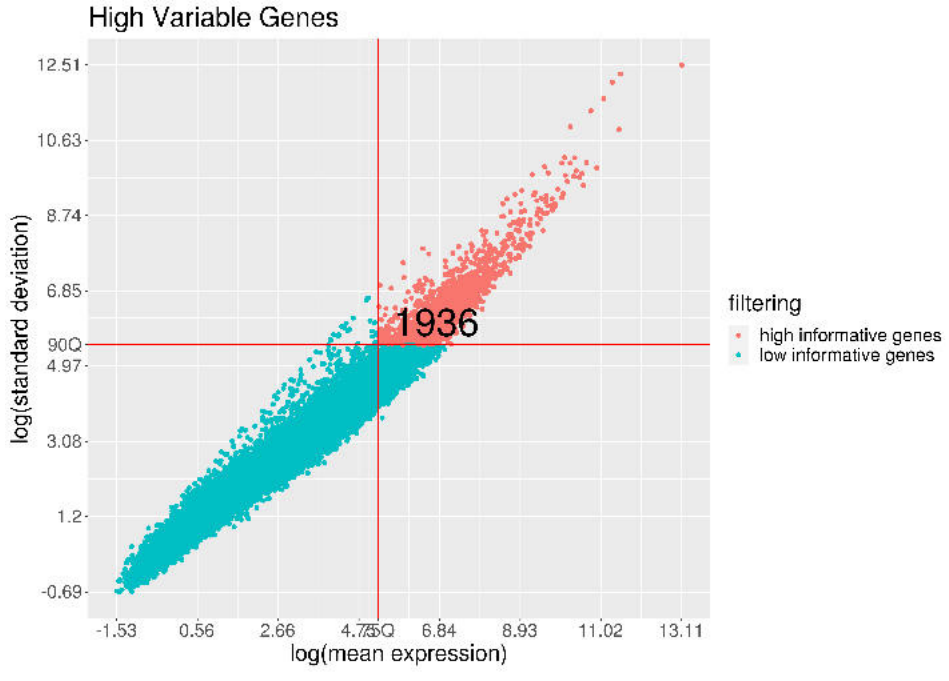
- Simoni N, Pavarana M, Micera R, Weindelmayer J, **Mengardo V**, Rossi G, Cenzi D, Tomezzoli A, Del Bianco P, Giacopuzzi S, De Manzoni G, Mazzarotto R. Long-Term Outcomes of Induction Chemotherapy Followed by Chemo-Radiotherapy as Intensive Neoadjuvant Protocol in Patients with Esophageal Cancer. Cancers (Basel). 2020 Dec 3;12(12):3614. doi: 10.3390/cancers12123614. PMID: 33287147; PMCID: PMC7761709.
- De Pasqual CA, **Mengardo V**, Tomba F, Veltri A, Sacco M, Giacopuzzi S, Weindelmayer J, de Manzoni G. Effectiveness of endoscopic vacuum therapy as rescue treatment in refractory leaks after gastro-esophageal surgery. Updates Surg. 2020 Nov 30. doi: 10.1007/s13304-020-00935-y. Epub ahead of print. PMID: 33258044.
- Simoni N, Rossi G, Benetti G, Zuffante M, Micera R, Pavarana M, Guariglia S, Zivelonghi E, **Mengardo V**, Weindelmayer J, Giacopuzzi S, de Manzoni G, Cavedon C, Mazzarotto R. ^{18}F-FDG PET/CT Metrics Are Correlated to the Pathological Response in Esophageal Cancer Patients Treated With Induction Chemotherapy Followed by Neoadjuvant Chemo-Radiotherapy. Front Oncol. 2020 Nov 27;10:599907. doi: 10.3389/fonc.2020.599907. PMID: 33330097; PMCID: PMC7729075.
- Bencivenga M, Torroni L, Verlato G, **Mengardo V**, Sacco M, Allum WH, de Manzoni G. Lymphadenectomy for gastric cancer at European specialist centres. Eur J Surg Oncol. 2020 Oct 15:S0748-7983(20)30845-3. doi: 10.1016/j.ejso.2020.10.013. Epub ahead of print. PMID: 33092970.
- Monti M, Morgagni P, Nanni O, Framarini M, Saragoni L, Marrelli D, Roviello F, Petrioli R, Fumagalli Romario U, Rimassa L, Bozzarelli S, Donini A, Graziosi L, De Angelis V, De Manzoni G, Bencivenga M, **Mengardo V**, Parma E, Milandri C, Mura G, Signorini A, Baiocchi G, Molfino S, Sgroi G, Steccanella F, Rausei S, Proserpio I, Viganò J, Brugnatelli S, Rinnovati A, Santi S, Ercolani G, Foca F, Valmorri L, Amadori D, Frassineti GL. Preoperative or Perioperative Docetaxel, Oxaliplatin, and Capecitabine (GASTRODOC Regimen) in Patients with Locally-Advanced Resectable Gastric Cancer: A Randomized Phase-II Trial. Cancers (Basel). 2020 Sep 29;12(10):E2790. doi: 10.3390/cancers12102790. PMID: 33003302; PMCID: PMC7599648.
- Hetoja S, De Pasqual CA, **Mengardo V**, Weindelmayer J, Giacopuzzi S. Gastric conduit perforation after Ivor Lewis esophagectomy successfully treated with endoscopic vacuum therapy (E-VAC): a case report. J Surg Case Rep. 2020 Aug 19;2020(8):rjaa251. doi: 10.1093/jscr/rjaa251. PMID: 32850114; PMCID: PMC7440394.
- De Pasqual CA, Torroni L, Gervasi MC, Alberti L, **Mengardo V**, Benedetti B, Giacopuzzi S, Weindelmayer J. Feasibility and safety of an enhanced recovery protocol (ERP) for upper GI surgery in elderly patients (≥ 75 years) in a high-volume surgical center. Updates Surg. 2020 Sep;72(3):751-760. doi: 10.1007/s13304-020-00824-4. Epub 2020 Jun 2. PMID: 32488821.
- Catalano F, Cerofolini A, **Mengardo V**, Tomezzoli A, Borghini R, Trecca A. Small type O-IIa de novo colon cancer treated with curative endoscopic full-thickness resection (EFTR). Tech Coloproctol. 2020 Jul;24(7):781-783. doi: 10.1007/s10151-020-02241-6. Epub 2020 May 22. PMID: 32444951.
- Weindelmayer J., **Mengardo V**, Veltri A, Baiocchi G, Giacopuzzi S, Verlato G, de Manzoni G. Utility of Abdominal Drain in Gastrectomy (ADiGe) Trial: study protocol for a multicenter non-inferiority randomized trial (TRLS-D-20-00745R2) accepted for publication in Trials 05.02.2021

- Weindelmayer J., **Mengardo V**, Gasparini A , Sacco M, Torroni L , Carlini M, Verlato G, de Manzoni G. Enhanced Recovery After Surgery can improve patients' outcomes and reduce hospital cost of gastrectomy for cancer in the West. A propensity score-based analysis. Under review in Annals of Surgical Oncology

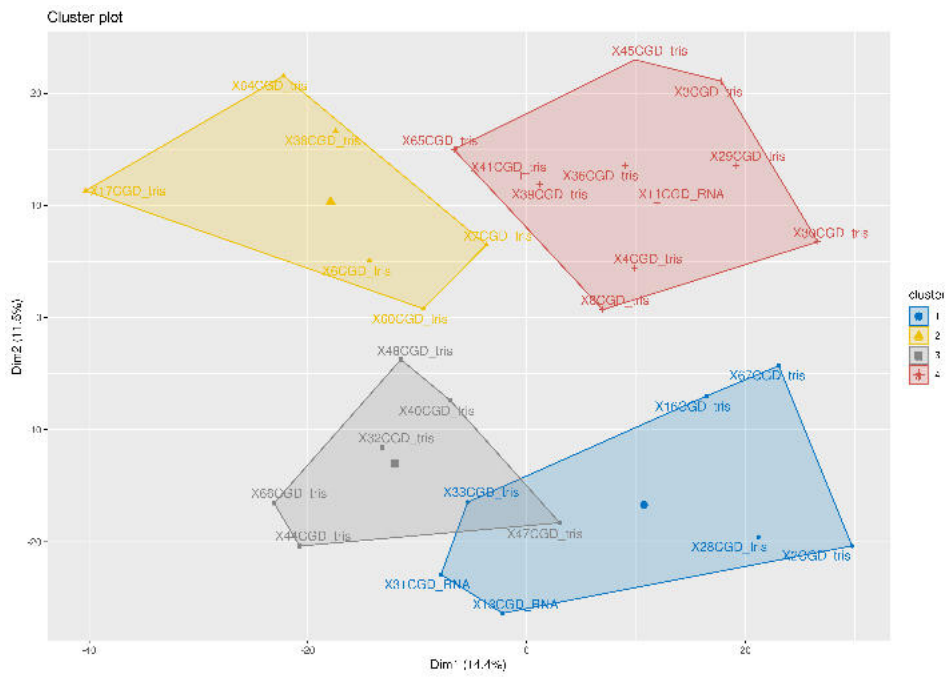
Il Responsabile Scientifico
Giovanni de Manzoni

L'Assegnista di Ricerca
Valentina Mengardo

A


High Variable Genes
12.51
10.63
8.74
6.85
90Q
4.97
3.08
1.2
-0.69
log(standard deviation)
1936
-1.53
0.56
2.66
4.75Q
6.84
8.93
11.02
13.11
log(mean expression)
filtering
high informative genes
low informative genes

B


Cluster plot
X45CGD_tris
X3CGD_tris
X65CGD_tris
X29CGD_tris
X41CGD_tris
X36CGD_tris
X38CGD_tris
X11CGD_RNA
X39CGD_tris
X4CGD_tris
X8CGD_tris
X64CGD_tris
X38CGD_tris
X17CGD_tris
X2CGD_tris
X6CGD_tris
X60CGD_tris
X48CGD_tris
X40CGD_tris
X32CGD_tris
X62CGD_tris
X16CGD_tris
X66CGD_tris
X33CGD_tris
X44CGD_tris
X47CGD_tris
X28CGD_tris
X26CGD_tris
X31CGD_RNA
X18CGD_RNA
cluster
1
2
3
4
Dim2 (11.5%)
Dim1 (14.1%)

C

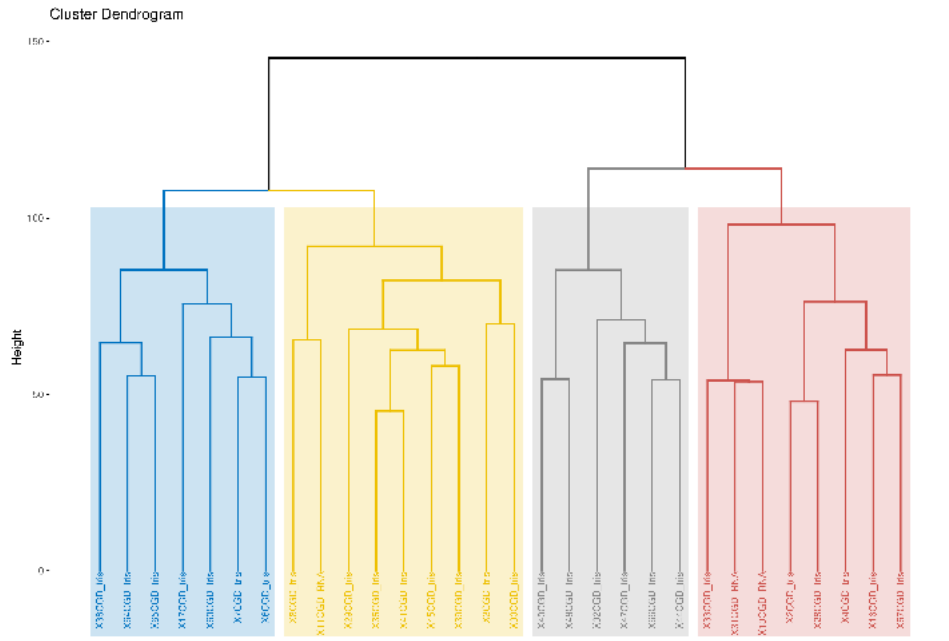

Cluster Dendrogram
150
100
50
0
Height

Figure 1.

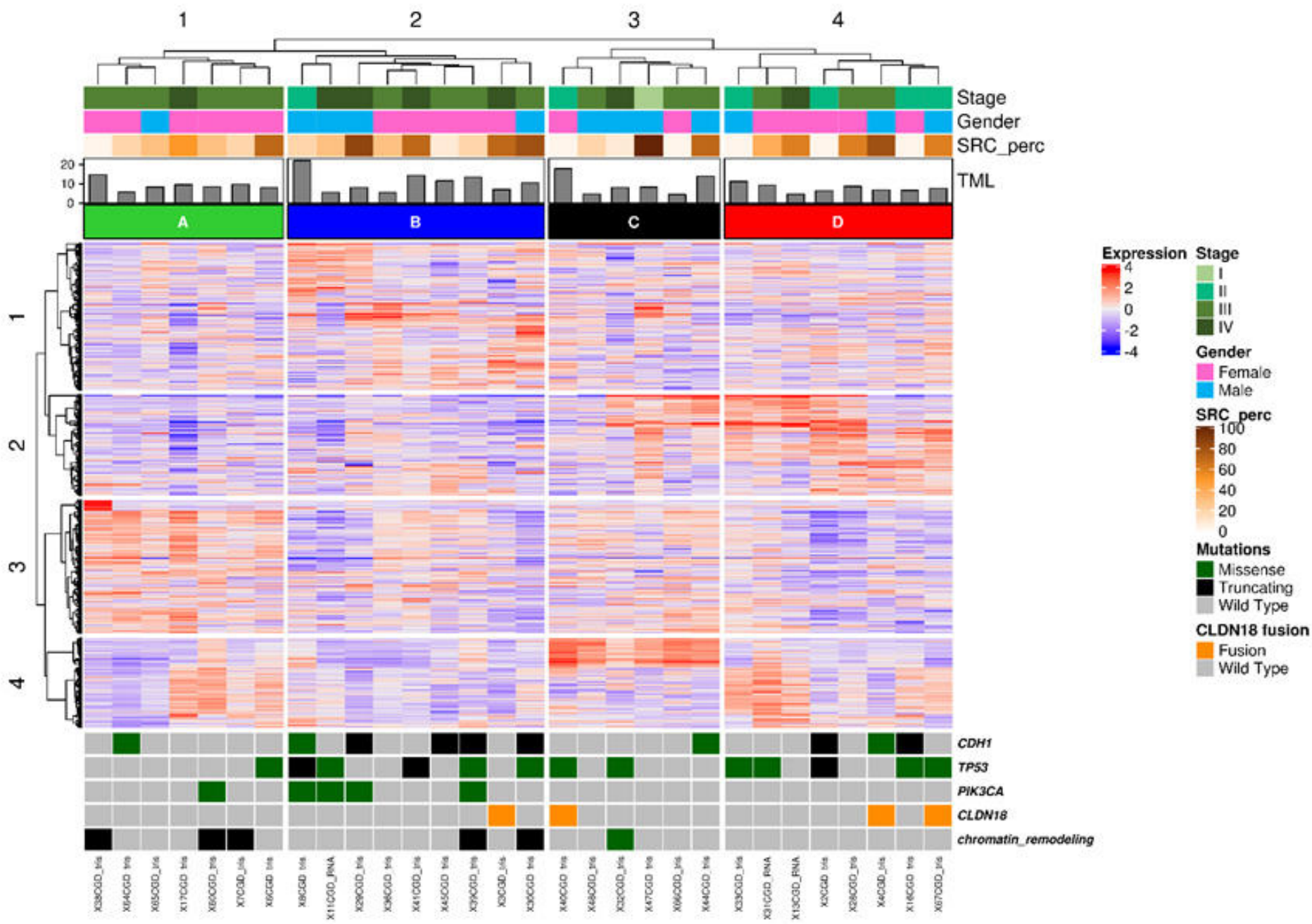
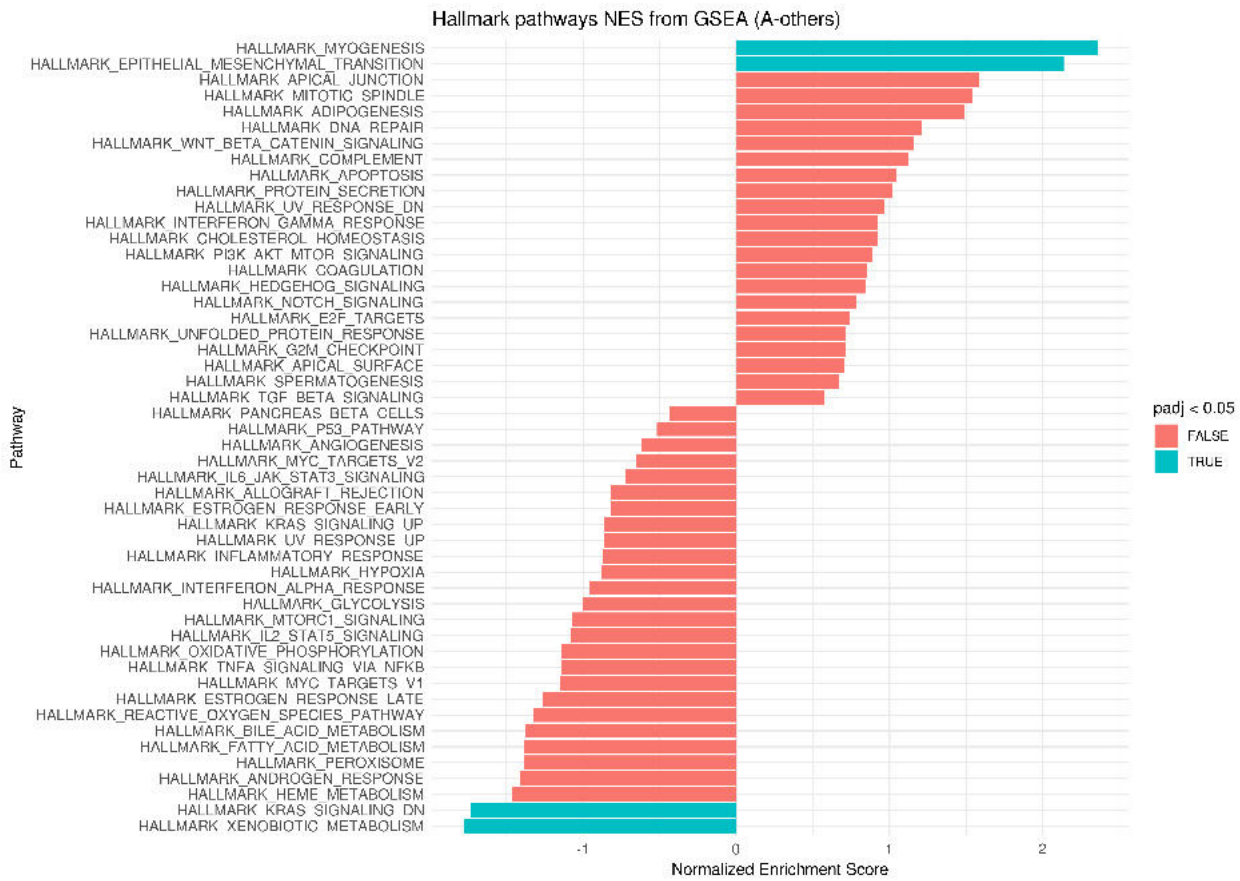
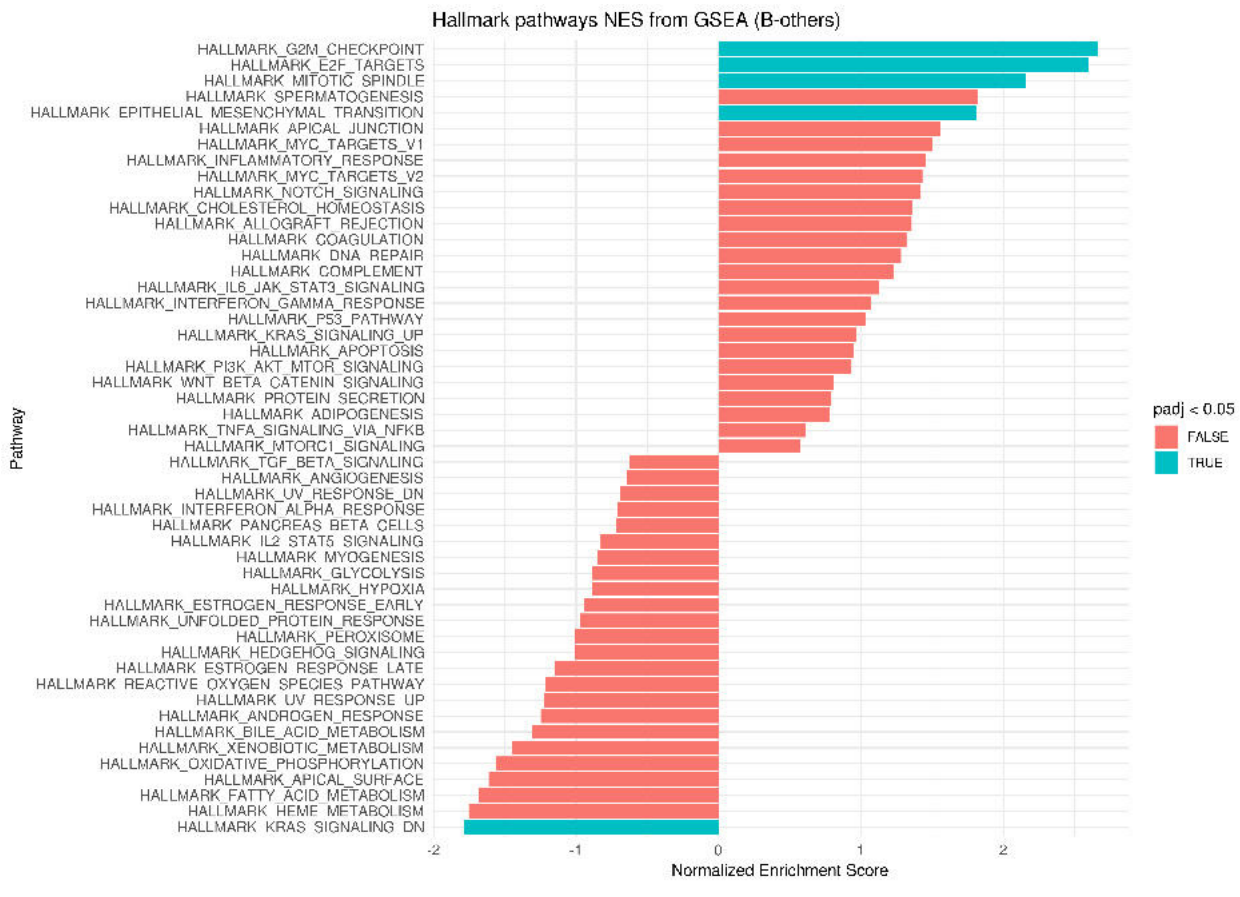

1
2
3
4
Stage
Gender
SRC_perc
TML
20
10
0
A
B
C
D
1
2
3
4
CDH1
TP53
PIK3CA
CLDN18
chromatin_remodeling
Expression
4
2
0
-2
-4
Stage
I
II
III
IV
Gender
Female
Male
SRC_perc
100
80
60
40
20
0
Mutations
Missense
Truncating
Wild Type
CLDN18 fusion
Fusion
Wild Type

Figure 2.

A


Hallmark pathways NES from GSEA (A-others)
HALLMARK_MYOGENESIS
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_APICAL_JUNCTION
HALLMARK_MITOTIC_SPINDLE
HALLMARK_ADIPOGENESIS
HALLMARK_DNA_REPAIR
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_COMPLEMENT
HALLMARK_APOPTOSIS
HALLMARK_PROTEIN_SECRETION
HALLMARK_UV_RESPONSE_DN
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_COAGULATION
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_NOTCH_SIGNALING
HALLMARK_E2F_TARGETS
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_G2M_CHECKPOINT
HALLMARK_APICAL_SURFACE
HALLMARK_SPERMATOGENESIS
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_PANCREAS_BETA_CELLS
HALLMARK_P53_PATHWAY
HALLMARK_ANGIOGENESIS
HALLMARK_MYC_TARGETS_V2
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_UV_RESPONSE_UP
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_HYPOXIA
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_GLYCOLYSIS
HALLMARK_MTORC1_SIGNALING
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_MYC_TARGETS_V1
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_PEROXISOME
HALLMARK_ANDROGEN_RESPONSE
HALLMARK_HEME_METABOLISM
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_XENOBIOTIC_METABOLISM
Pathway
-1
0
1
2
Normalized Enrichment Score
padj < 0.05
FALSE
TRUE

B


Hallmark pathways NES from GSEA (B-others)
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
HALLMARK_MITOTIC_SPINDLE
HALLMARK_SPERMATOGENESIS
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_APICAL_JUNCTION
HALLMARK_MYC_TARGETS_V1
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_MYC_TARGETS_V2
HALLMARK_NOTCH_SIGNALING
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_COAGULATION
HALLMARK_DNA_REPAIR
HALLMARK_COMPLEMENT
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_P53_PATHWAY
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_APOPTOSIS
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_PROTEIN_SECRETION
HALLMARK_ADIPOGENESIS
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_MTORC1_SIGNALING
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_ANGIOGENESIS
HALLMARK_UV_RESPONSE_DN
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_PANCREAS_BETA_CELLS
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_MYOGENESIS
HALLMARK_GLYCOLYSIS
HALLMARK_HYPOXIA
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_PEROXISOME
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY
HALLMARK_UV_RESPONSE_UP
HALLMARK_ANDROGEN_RESPONSE
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_XENOBIOTIC_METABOLISM
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_APICAL_SURFACE
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_HEME_METABOLISM
HALLMARK_KRAS_SIGNALING_DN
Pathway
-2
-1
0
1
2
Normalized Enrichment Score
padj < 0.05
FALSE
TRUE

C

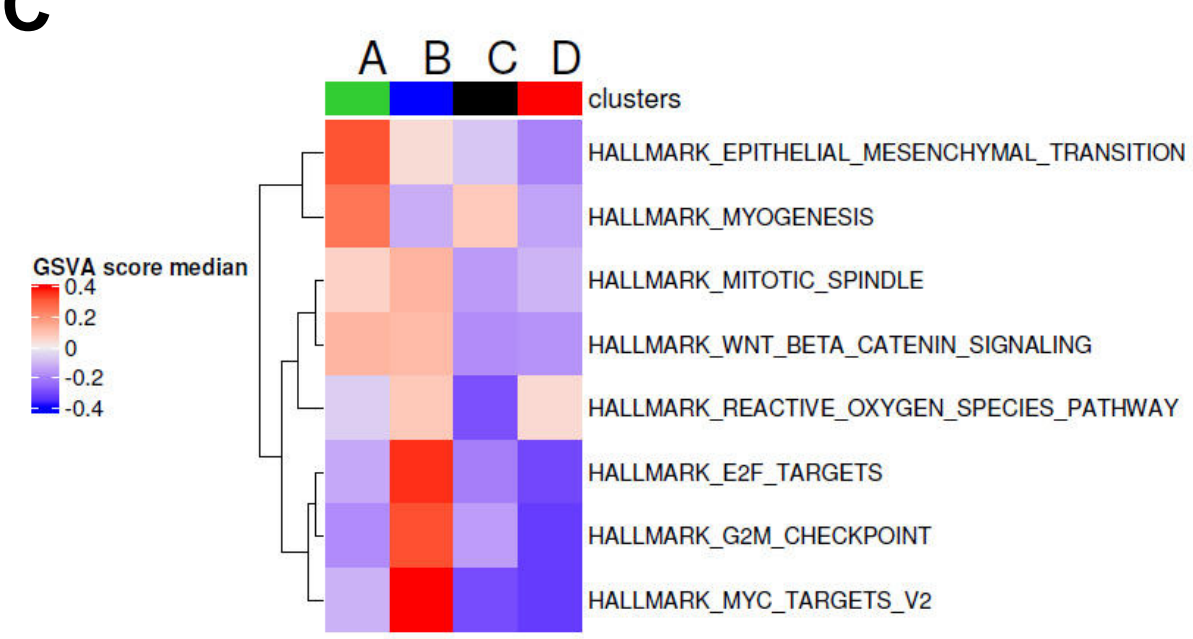
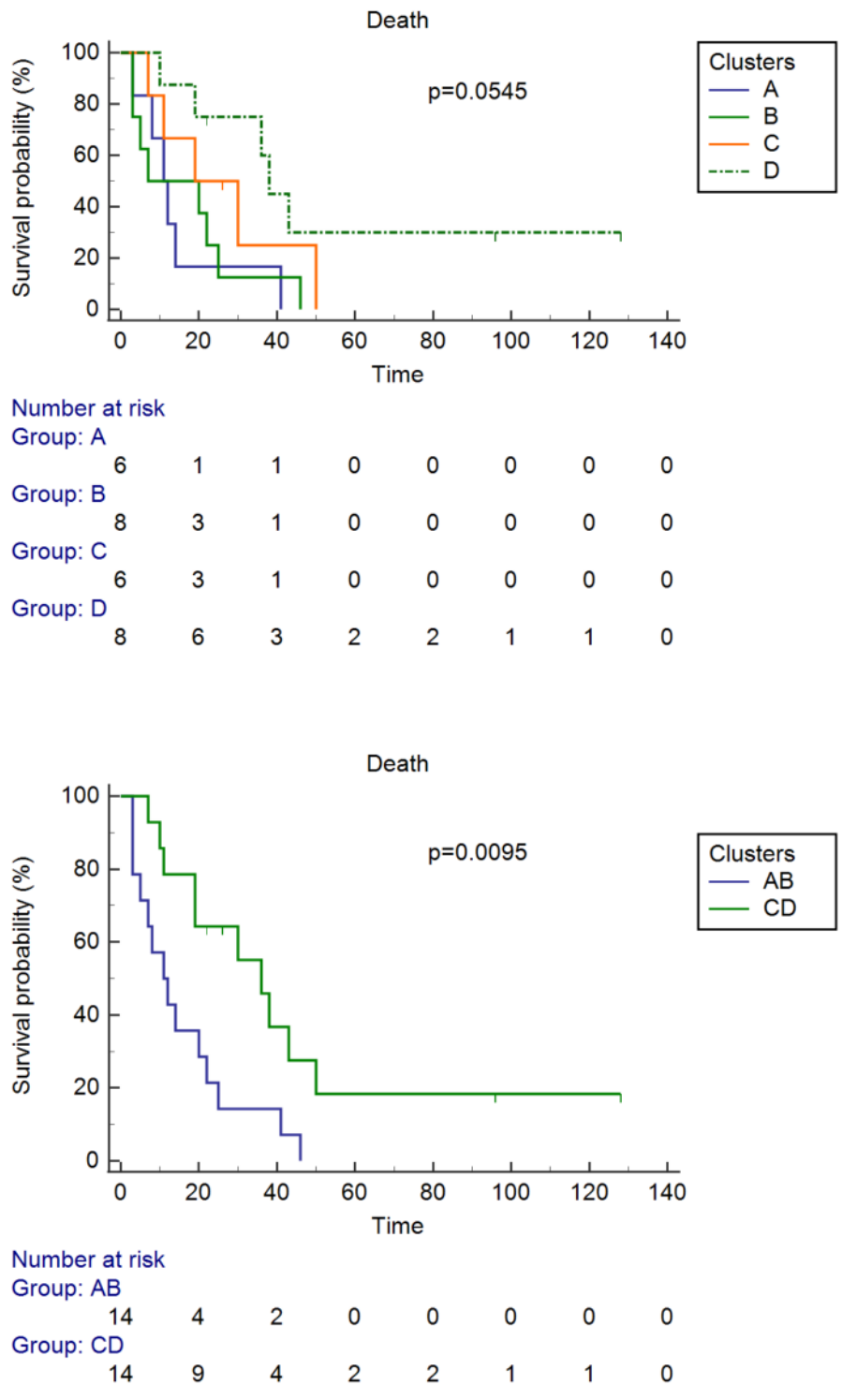

A
B
C
D
clusters
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_MYOGENESIS
HALLMARK_MITOTIC_SPINDLE
HALLMARK_WNT_BETA_CATENIN_SIGNALING
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY
HALLMARK_E2F_TARGETS
HALLMARK_G2M_CHECKPOINT
HALLMARK_MYC_TARGETS_V2
GSVA score median
0.4
0.2
0
-0.2
-0.4

Figure 3.

A


Death
p=0.0545
Survival probability (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
140
Time
Clusters
A
B
C
D
Number at risk
Group: A
6 1 1 0 0 0 0 0
Group: B
8 3 1 0 0 0 0 0
Group: C
6 3 1 0 0 0 0 0
Group: D
8 6 3 2 2 1 1 0

B

Death
p=0.0095
Survival probability (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
140
Time
Clusters
AB
CD
Number at risk
Group: AB
14 4 2 0 0 0 0 0
Group: CD
14 9 4 2 2 1 1 0

Figure 4.

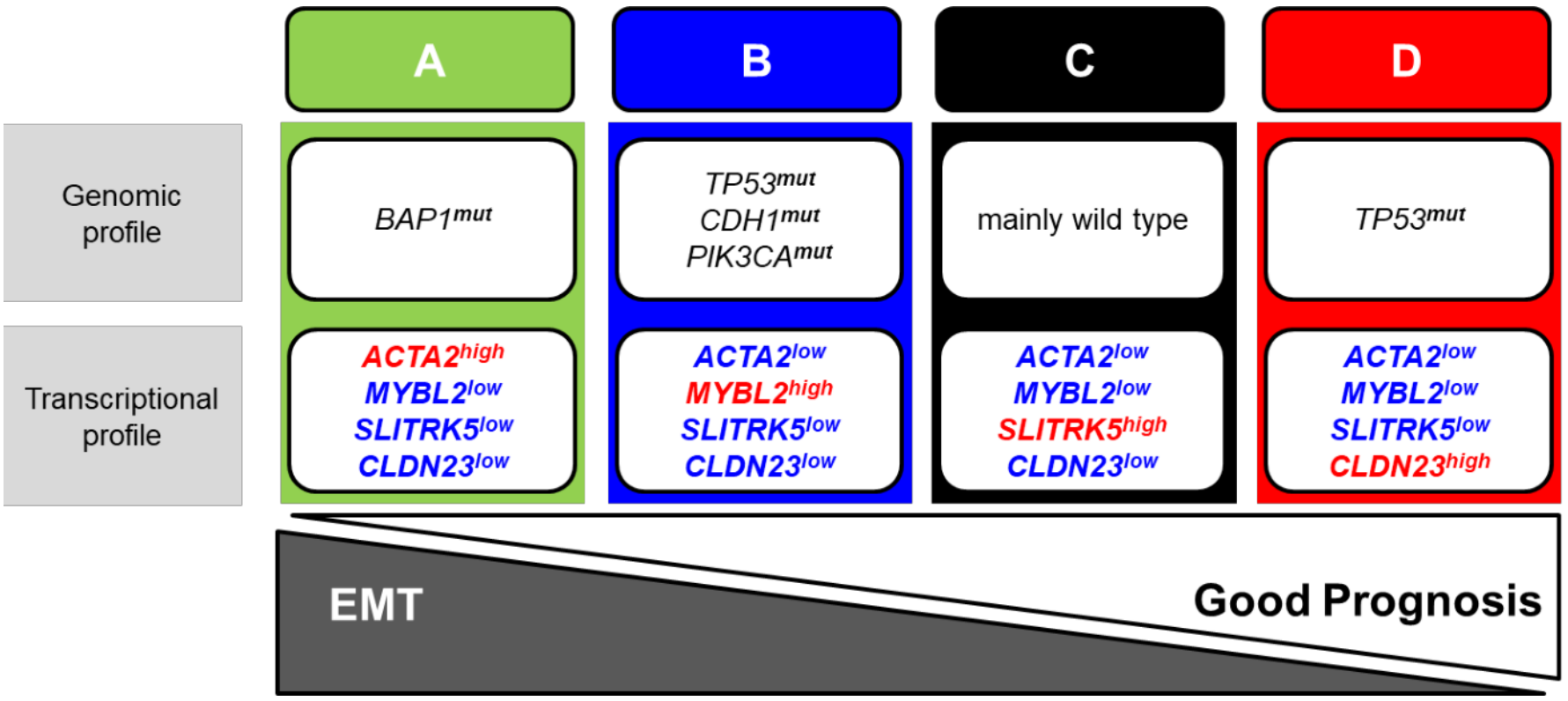

A
B
C
D
Genomic profile
BAP1mut
TP53mut
CDH1mut
PIK3CAmut
mainly wild type
TP53mut
Transcriptional profile
ACTA2high
MYBL2low
SLITRK5low
CLDN23low
ACTA2low
MYBL2high
SLITRK5low
CLDN23low
ACTA2low
MYBL2low
SLITRK5high
CLDN23low
ACTA2low
MYBL2low
SLITRK5low
CLDN23high
EMT
Good Prognosis

Figure 5.