COMPREHENSIVE GENOMIC CHARACTERIZATION OF A LARGE COHORT OF PLATINUM-SENSITIVE, HIGH-GRADE SEROUS OVARIAN CANCER (HGSOC) FFPE SPECIMENS

Abstract # 1743

Brian C Haynes[1], Marie E Fahey[1], Darcy Myers[1], Diane Ilsley[1], Gary J Latham[1], Elizabeth B Somers[2], Nicholas C Nicolaides[2], Charles Schweizer[2], Daniel J O'Shannessy[2]
[1]Asuragen, Inc., Austin, TX, [2]Morphotek, Inc., Exton, PA

SUMMARY

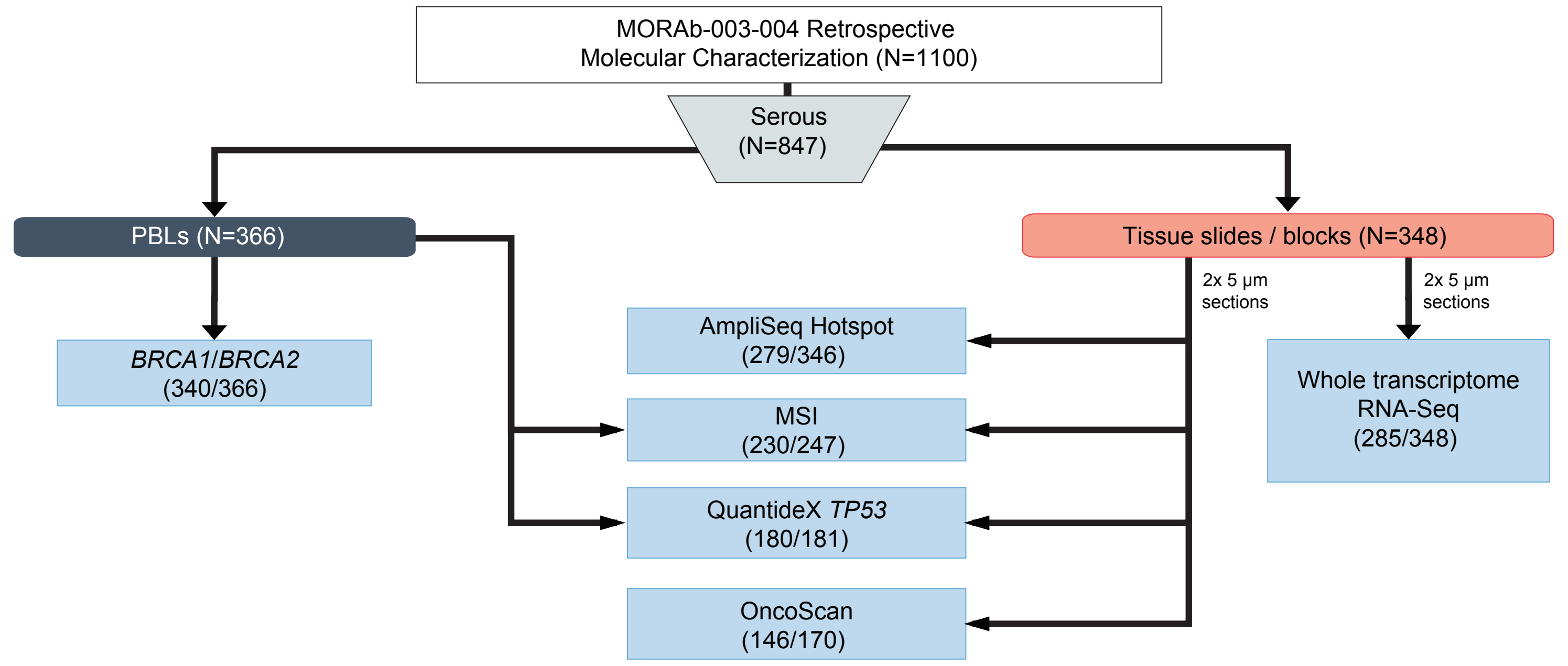
- A broad retrospective molecular characterization of 348 FFPE platinum-sensitive HGSOC tumors was performed.
- Analysis of DNA mutations and CNVs revealed recurrent variations consistent with the TCGA HGSOC cohort.
- Transcriptional subtyping results were concordant with fresh-frozen HGSOC cohorts and reproduced the CLOVAR gene signature.

INTRODUCTION

Ovarian cancer is a leading cause of cancer related death in women. A comprehensive genomic characterization of platinum-sensitive tumors is required to further refine the definition of molecular subtypes and identify targeted therapies for this patient population. To this end we have performed a large-scale genomic and transcriptomic retrospective analysis of 348 FFPE tissues from a cohort of platinum-sensitive HGSOC patients collected from over 100 clinical sites.

MATERIALS AND METHODS

FFPE tumors and matched PBL specimens were sourced from the MORAb-003-004 clinical trial. Macrodissection of FFPE resected tumor slides was performed to enrich for tumor content. RNA expression was profiled by whole transcriptome RNA-Seq. DNA variants were analyzed by the AmpliSeq™ Cancer Hotspot Panel (Thermo Fisher). A subset of tumor and matched germline (PBL) specimens (N=181) were assessed for *TP53* mutations by the QuantideX® NGS *TP53* Assay (Asuragen, Inc.). CNV analysis of FFPE tumor DNA was performed with the OncoScan® FFPE Assay Kit (Affymetrix) and microsatellite instability was characterized by comparing matched tumor and PBL specimens with PCR/CE using the Bethesda panel. Germline *BRCA* mutation status was determined by profiling PBL specimens with a custom AmpliSeq™ NGS panel.



MORAb-003-004 Retrospective
Molecular Characterization (N=1100)

Serous
(N=847)

PBLs (N=366)

BRCA1/BRCA2
(340/366)

Tissue slides / blocks (N=348)

2x 5 μm
sections

2x 5 μm
sections

AmpliSeq Hotspot
(279/346)

MSI
(230/247)

QuantideX *TP53*
(180/181)

OncoScan
(146/170)

Whole transcriptome
RNA-Seq
(285/348)

Figure 1. Summary of retrospective molecular characterization of MORAB-003-004. Fractions in parentheses indicate number of patients for which data was obtained/number of patients attempted. Assays were sequentially performed on the resulting isolations where material permitted.

RESULTS

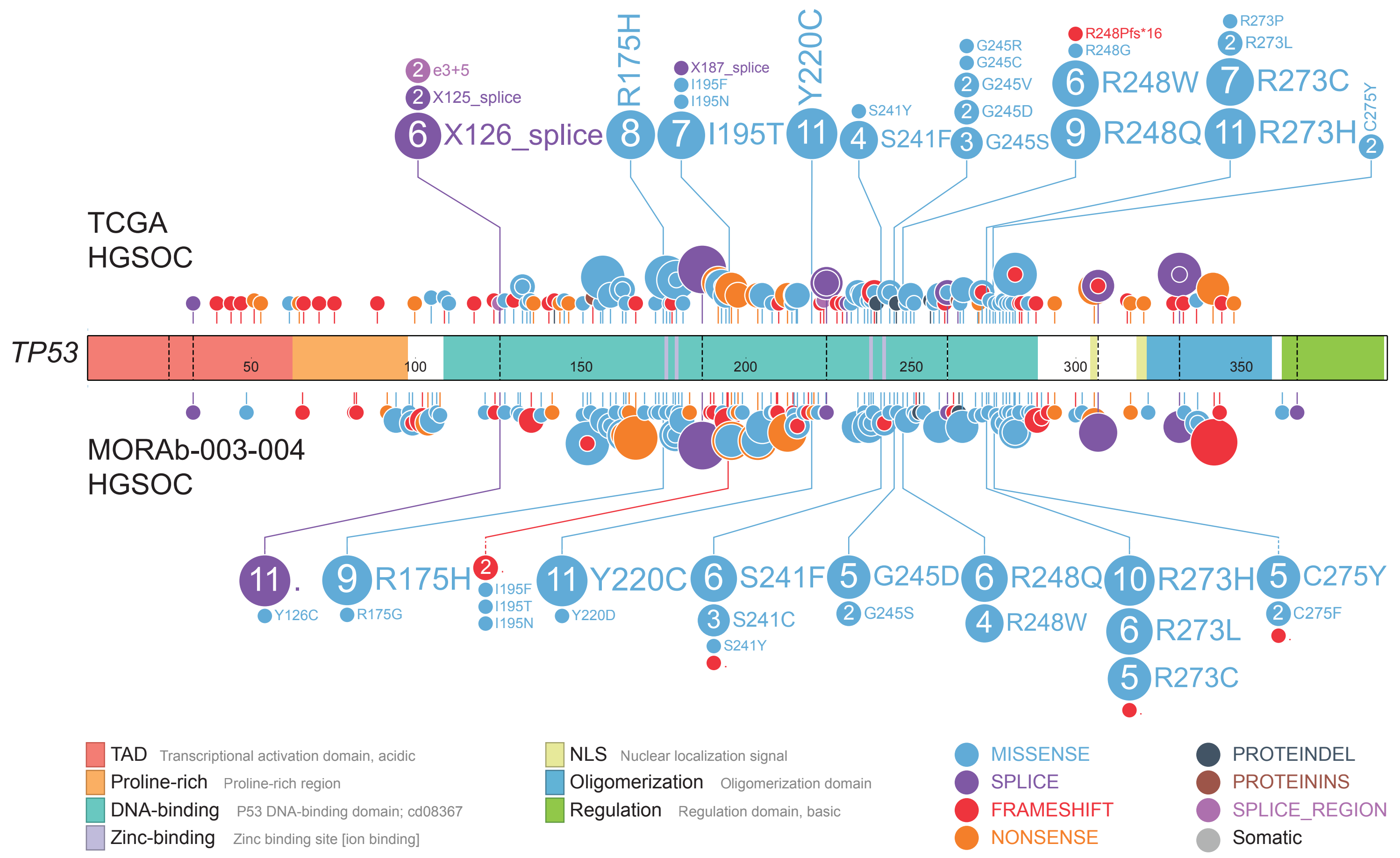


Figure 2. Mutational spectrum of *TP53* was concordant with TCGA HGSOC cohort. *TP53* mutations were present in the majority of subjects (87% were *TP53* positive for cases with full exon coverage of *TP53*).

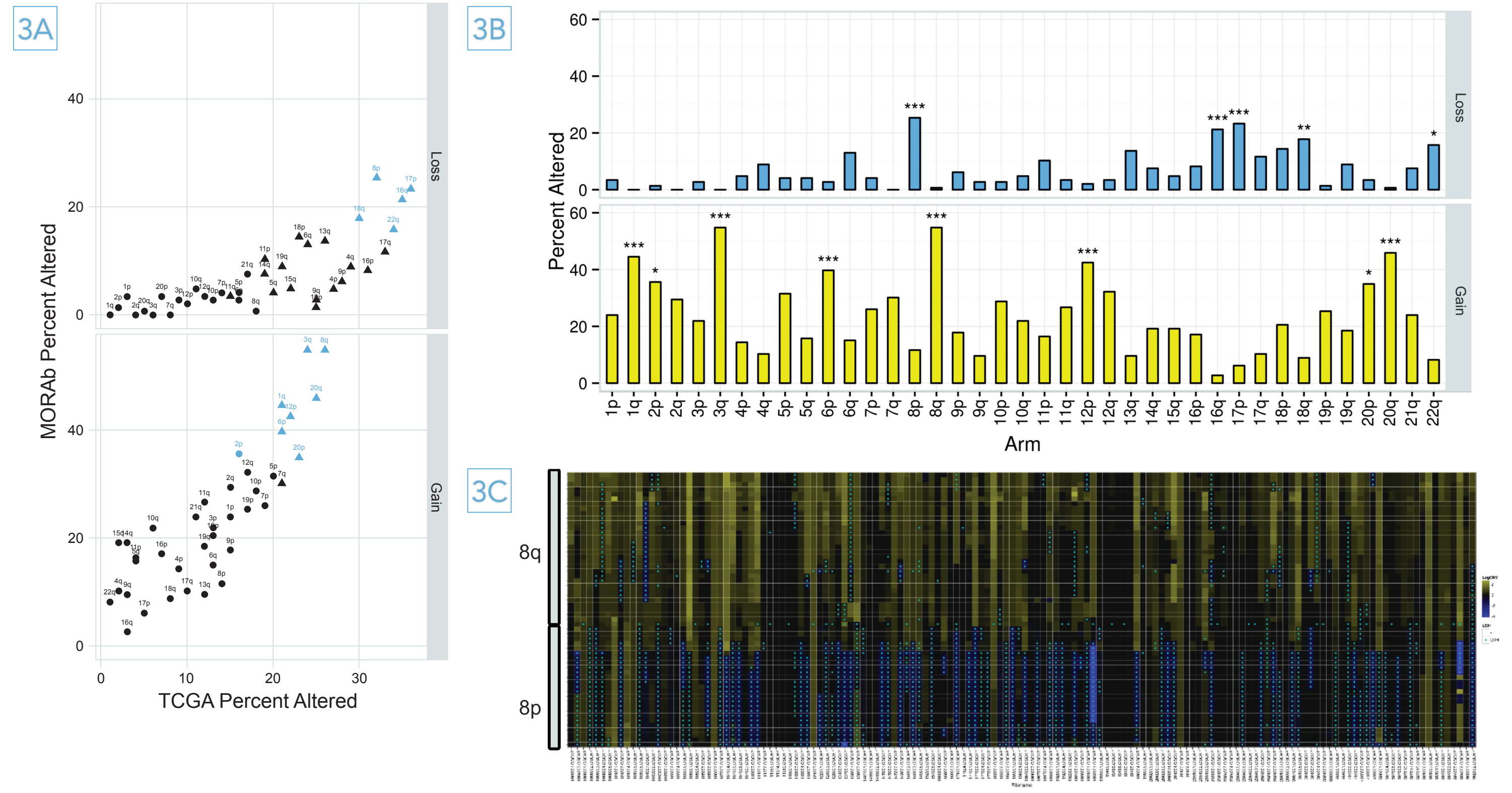


Figure 3. A) Concordance of whole-arm loss (top) and gain (bottom) events with the TCGA HGSOC cohort. Significant events in MORAb-003-004 in blue and significant events in TCGA distinguished as triangles. **B)** Frequency of whole arm gain and loss events in MORAb-003-004 with significantly frequent events indicated: *(<0.05) **(<0.01) ***(<0.001). **C)** Visualization of gain (yellow) and loss (blue) events across chromosome 8 for 146 subjects (x-axis). Blue dots indicate loss of heterozygosity, which is concomitant with copy number loss events.

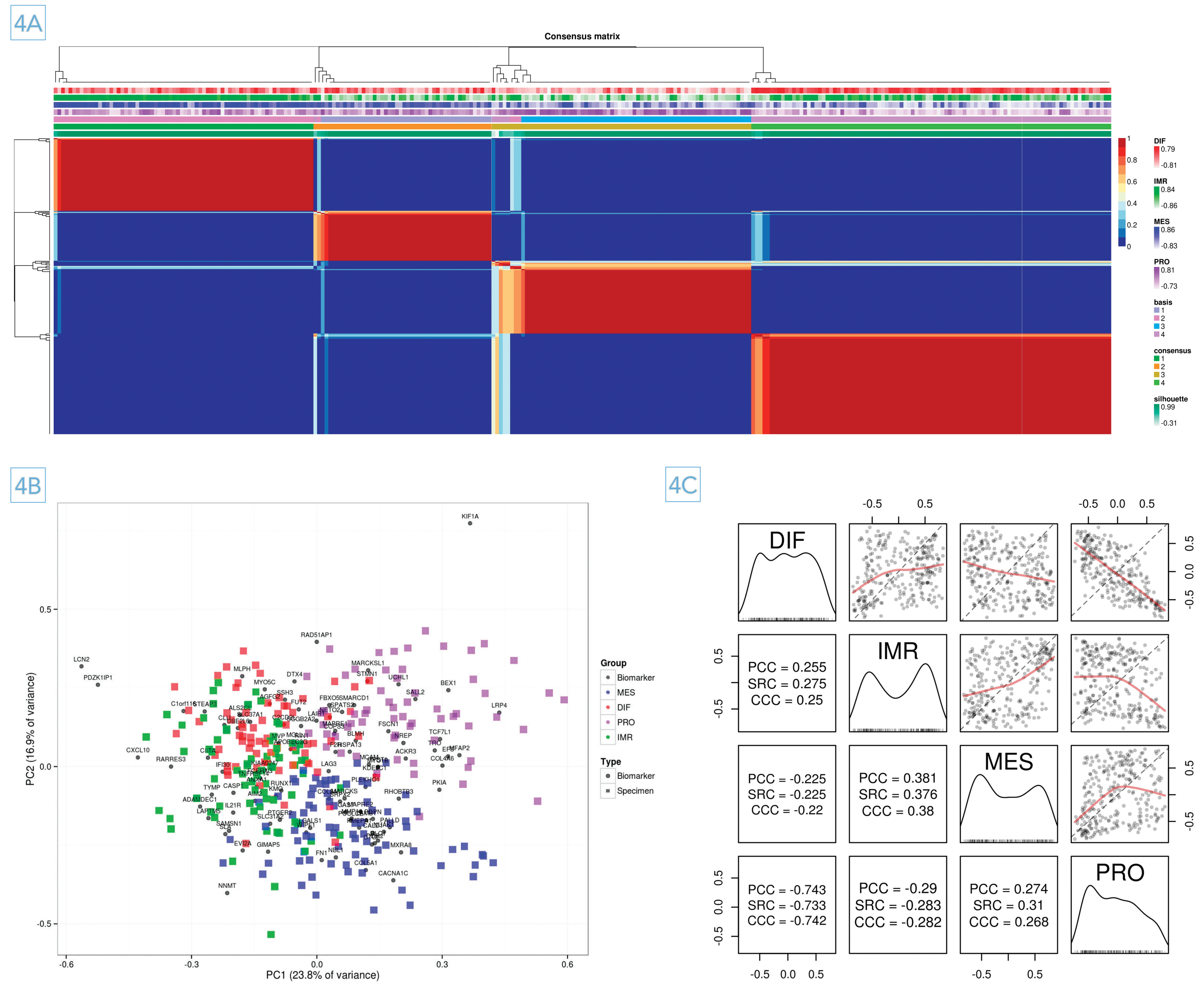


Figure 4. A) Unsupervised clustering by non-negative matrix factorization of whole transcriptome RNA-Seq data identified 4 distinct expression subtypes which corresponded to the established CLOVAR subtypes. **B)** The 100 gene CLOVAR signature clustered the cohort into four distinct molecular subtypes by PCA: differentiated (DIF), immunoreactive (IMR), mesenchymal (MES) and proliferative (PRO). **C)** Gene set variation analysis (GSVA) score correlations across the 4 subtypes revealed a negative correlation between differentiated and proliferative signatures.

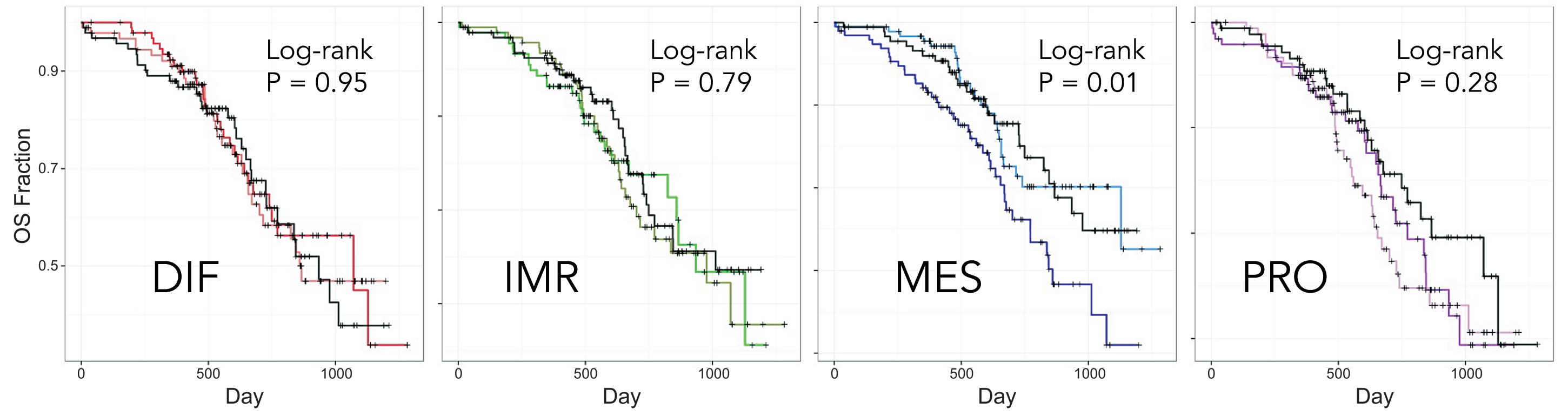


Figure 5. Consistent with TCGA and other HGSOC studies, the CLOVAR mesenchymal (MES) subtype was negatively prognostic (all subjects analyzed independent of treatment). KM analysis of GSVA score tertiles showed poorer overall survival for subjects with higher MES scores. Higher score tertiles indicated by color saturation for each respective subtype.

Fusion	Recurrence	Type	Mitelman status
RPL22/MECOM	20	Interchromosomal	3p
NF1/AK4	15	Interchromosomal	5p
ZCCHC14/RERE	8	Interchromosomal	3p
SPECC1/CCDC144A	7	Intrachromosomal	5p
BANP/SNX29	4	Intrachromosomal	3p
GNG10/UGCG	4	Intrachromosomal	3p
EIF1AX/PDE4DIP	3	Interchromosomal	3p
AFF3/RGPD5	3	Intrachromosomal	5p
COL14A1/DEPTOR	3	Intrachromosomal	5p, 3p
AKAP8L/BRD4	2	Intrachromosomal	3p
FN1/NRG1	2	Interchromosomal	3p
LRRC37A/WDR55	2	Interchromosomal	3p
NEO1/WDR72	2	Intrachromosomal	3p
SAMD5/SASH1	2	Intrachromosomal	3p
GNB1/CFAP74	2	Intrachromosomal	5p
ITCH/FRG1	2	Interchromosomal	5p
SLC29A1/HSP90AB3P	2	Interchromosomal	5p
CTNNA1/MATR3	2	Intrachromosomal	5p, 3p
PLXND1/RPN1	2	Intrachromosomal	5p, 3p
CCDC6/ANK3	2	Intrachromosomal	Known

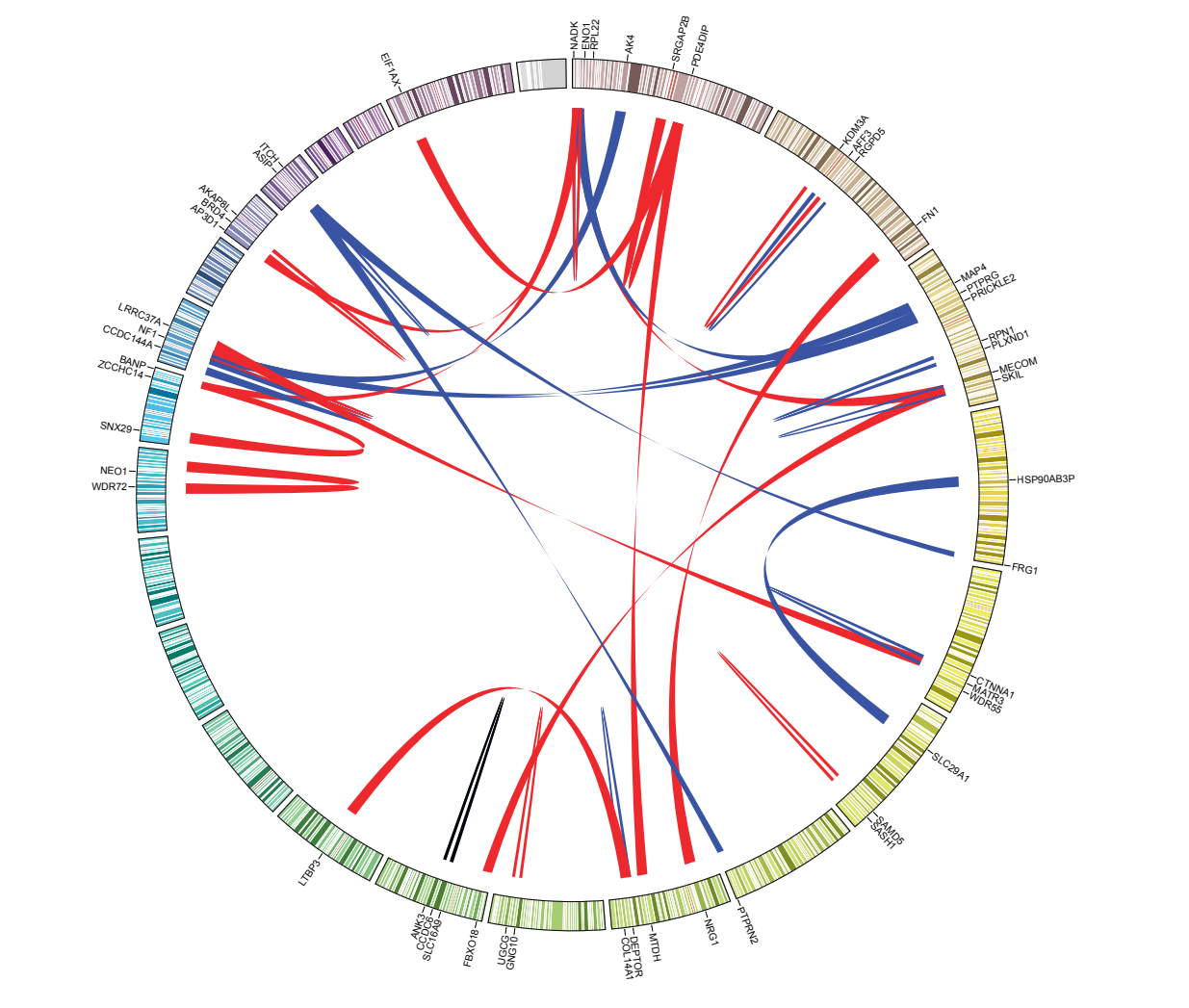


Figure 6. RNA-Seq analysis identified gene fusions with 5′ (blue) and 3′ (red) partners reported in the Mitelman CGAP database. Twenty fusions were recurrent (present in two or more specimens). The two most frequently fused genes *MECOM* and *NF1* are also reported as recurrently focally amplified in HGSOC by TCGA. Other fusions such as *CCDC6-ANK3* (black) have been previously reported in breast and ovarian cancer.

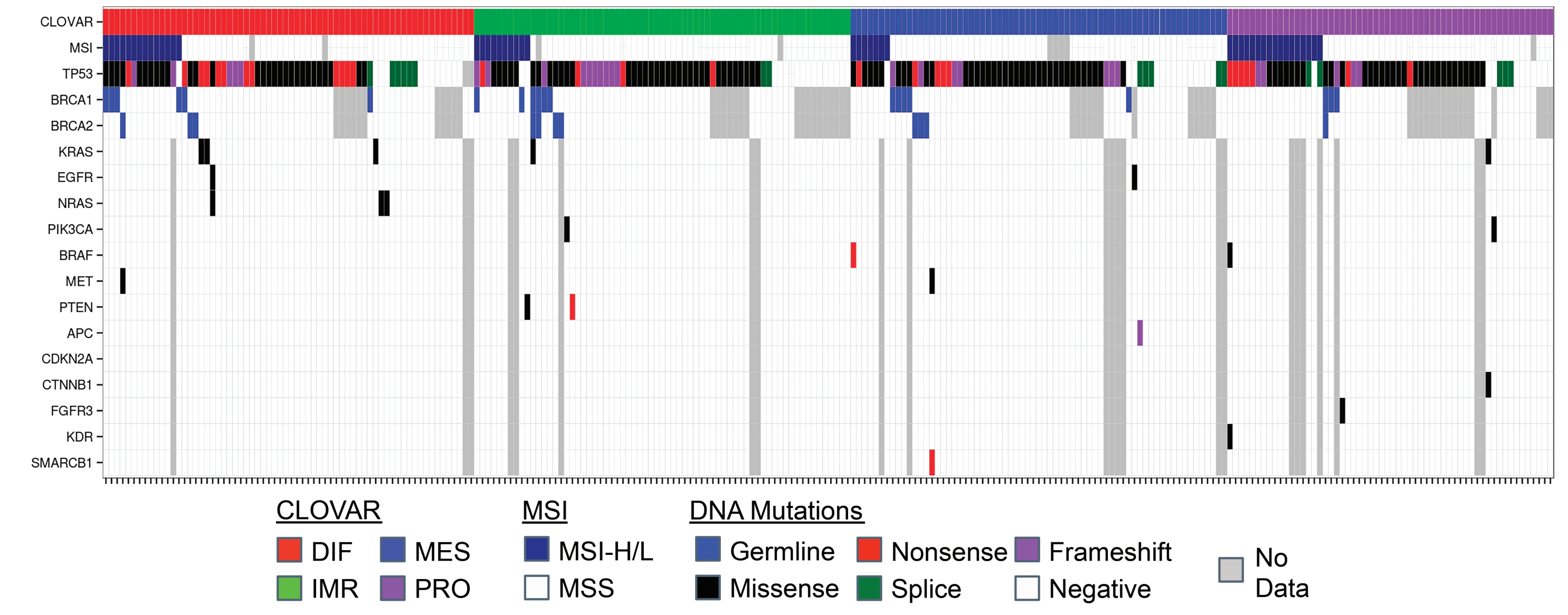


Figure 7. Landscape of molecular indications in 258 serous ovarian carcinomas covered by DNA and RNA sequencing including transcriptional subtyping by CLOVAR, DNA variant analysis, and microsatellite instability.

CONCLUSIONS

- The CLOVAR signature was reproduced in a cohort of FFPE HGSOC tumors with a previously reported association between the mesenchymal subtype and poor prognosis.
- The spectrum of DNA mutations, CNVs, gene fusions, and transcriptional subtypes affirms the representative nature of this HGSOC cohort.
- The study further serves as a model for extracting known and novel tumor biology through conservative use of FFPE tissue collected from real-world clinical settings.

Presented at AACR 2017

morphotek

Asuragen
www.asuragen.com