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Whole exome sequencing of low grade serous ovarian carcinoma identifies genomic events associated with clinical outcome

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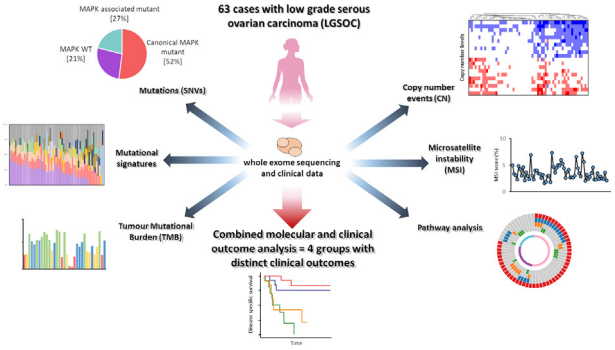
HIGHLIGHTS

- We present the largest whole exome sequencing cohort of clinically annotated LGSOC.
- 52% harbour canonical MAPK mutations, 27% have mutations in other MAPK associated genes and only 21% are MAPK wild-type
- Tumour mutational burden, mutational signatures and copy number perturbations at chromosome 1 are frequent across the cohort.
- Combined analysis of genomic features identifies four classes with distinct genomic properties and clinical outcomes.
- LGSOC patients with low mutational burden and chromosome 1 copy number disruption demonstrate notably inferior survival.

GRAPHICAL ABSTRACT



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ABSTRACT

Objectives. Low-grade serous ovarian carcinoma (LGSOC) is a distinct, rare, ovarian cancer type characterised by younger patient age and intrinsic chemoresistance. Understanding the molecular landscape is crucial for optimising targeted therapy.

Methods. Genomic data from whole exome sequencing of tumour tissue was analysed in a LGSOC cohort with detailed clinical annotation.

Results. 63 cases were analysed and three subgroups identified based on single nucleotide variants: canonical MAPK mutant (cMAPKm: 52%, *KRAS*/*BRAF*/*NRAS*), MAPK-associated gene mutation (MAPK-assoc: 27%) and

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MAPK wild-type (MAPKwt: 21%). NOTCH pathway disruption occurred across all subgroups. Tumour mutational burden (TMB), mutational signatures and recurrent copy number (CN) changes varied across the cohort with co-occurrence of chromosome 1p loss and 1q gain (CN Chr1pq) a recurrent feature. Low TMB and CN Chr1pq were associated with inferior disease-specific survival (HR 6.43; $p < 0.001$ and HR 3.29, $p = 0.011$ respectively). Stepwise genomic classification in relation to outcome resulted in four groups (TMB low; CN Chr1pq; MAPKwt/MAPKassoc; cMAPKm). 5 year disease-specific survival was 46%, 55%, 79% and 100% respectively for these groups. The two most favourable genomic subgroups were enriched for the SBS10b mutational signature, particularly the cMAPKm subgroup.

Conclusions. LGSOC comprises multiple genomic subgroups with distinct clinical and molecular features. Chr1pq CN arm disruption and TMB represent promising methods to identify individuals with poorer prognosis. Further investigation of the molecular basis for these observations is required. MAPKwt cases represent around a fifth of patients. NOTCH inhibitors represent a candidate therapeutic strategy worthy of exploration across these cases.

1. Background

Low grade serous ovarian carcinoma (LGSOC) is an uncommon and under-investigated ovarian cancer type, representing ≤5% of cases and arises from serous borderline tumours in a subset of patients [1]. LGSOC often affects younger women (median age at diagnosis 46–50 years) [2] and the response rate to platinum-based chemotherapy is low (5–25%) compared to high grade serous ovarian carcinoma (HGSOC, 70–80%) [3,4]. Relapsed LGSOC often follows a more indolent course compared to HGSOC, demonstrating prolonged post-relapse survival [5,6]. Also in contrast to HGSOC, LGSOC is ubiquitously *TP53* wild-type, does not demonstrate extensive genomic instability or homologous recombination repair deficiency, and frequently harbours classical activating mutations in canonical MAPK pathway components (*KRAS*, *BRAF* and *NRAS*) [7–11].

The appreciation of LGSOC as a separate entity has driven a clinical management shift away from the conventional 'one size fits all' approach to ovarian cancer treatment. Endocrine therapy shows significant activity in this disease setting [8,12], with the majority of LGSOC displaying high oestrogen receptor (ER) expression, and a subset demonstrating high progesterone receptor (PR) expression [13]. Recently, the MEK inhibitor trametinib also demonstrated notable efficacy, significantly delaying disease progression in the context of LGSOC recurrence compared to standard of care therapies, with an objective response rate of 26% [14]. Another MEK inhibitor, binimetinib, while not demonstrating superiority to standard of care chemotherapy, demonstrated an overall response rate of 16% [15]. In both of these MEK inhibitor studies, translational analysis of specimens has suggested that those with activating mutations in canonical MAPK pathway components may be more likely to respond (44% response rate to binimetinib in *KRAS* mutant [15], 50% response rate to trametinib in *KRAS*/*NRAS*/*BRAF* mutant [14]).

Until recently, genomic characterisation of LGSOC had been limited to targeted sequencing of selected genes in LGSOC cohorts of modest size [9,10,16] or whole exome sequencing in small numbers of cases ($n = 8$, $n = 9$, $n = 22$) [10,11,17]. Within the last three years, a number of studies analysing larger cohorts by targeted sequencing have been reported [7,18,19]. Collectively, these recent studies have demonstrated *KRAS*, *NRAS* and *BRAF* mutation rates of around 33%, 10% and 10% in LGSOC. Moreover, it has become apparent that cases with canonical MAPK component mutations (most notably *KRAS*), may experience more favourable survival [9,18,19]. However, the number of unselected comprehensive genomically characterised (whole exome or whole genome sequencing) LGSOC specimens remains low. Previous studies have also highlighted a number of mutations in genes associated with the MAPK pathway which are not canonical MAPK components (e.g. *NF1*); it remains unclear whether these cases more closely resemble a MAPK mutant or MAPK wild-type population.

A comprehensive analysis of the molecular landscape in LGSOC presents the opportunity to better define patients for whom currently available strategies may be most effective, optimise the use of molecular therapies, identify novel therapeutically exploitable biology in patients least well served by available regimes, and better characterise the relationship between molecular features and clinical phenotypes.

Here we perform comprehensive genomic characterisation of robustly curated LGSOC specimens by whole exome sequencing, including the analysis of single-nucleotide variants (SNVs), mutational signatures and copy number (CN) events to define a higher resolution landscape in this tumour type. In addition to canonical MAPK mutations (*KRAS, NRAS & BRAF),* we identify additional samples containing other mutations in the MAPK pathway. Through combined analysis of genomic data we identify genomic classes demonstrating differing survival outcomes and highlight high-risk groups for which new treatment strategies are required to improve outcome.

2. Methods

2.1. Ethical approval

Ethical approval was obtained from the Lothian Human Annotated Bioresource (reference 15/ES/0094-SR925), NKI-AVL Translational Research Board (NKI: Netherlands Cancer Institute. Reference CFMPB284), and University of Amsterdam AMC (Academic Medical Center) Biobank Assessment Committee (reference 2016_070#A201641). All participants provided written informed consent or had consent waived by the ethics committee due to the retrospective nature of the study.

2.2. Cohort identification and pathology review

Primary low grade serous ovarian cancer cases were identified in Edinburgh, UK and Amsterdam, Netherlands. For the Edinburgh cohort, all cases with a documented diagnosis of low grade or well differentiated (grade I) serous carcinoma on the Edinburgh Ovarian Cancer Database [6] were considered for the study ($n = 116$). AMC and NKI cases were identified from local pathology databases, using grade I serous, low grade serous and serous borderline tumour as diagnosis search terms ($n = 56$ and $n = 84$, respectively). 204 cases had material available for pathology review (Fig. S1). All cases underwent expert gynaecological pathology review by two independent pathologists (KvdV, CSH), with the assistance of WT1 and p53 immunohistochemistry in every case. Inclusion criteria for the study were: WT1 positive, p53 wild-type staining LGSOC histology with definitive stromal invasion, sufficient material for exome sequencing and wild-type *TP53* sequence in the resulting data. 86 cases were excluded during pathology review, including all WT1 negative or p53 aberrant cases (61 borderline/no definitive invasion, 1 cystadenoma, 23 non-LGSOC carcinomas, 1

uninterpretable WT1 stain); of the remaining cases, 44 were excluded prior to whole exome sequencing (WES; 9 insufficient material, 32 low cellularity, 3 failed DNA quality control). 11 were excluded following WES (5 low sequencing coverage, 6 *TP53* mutant. Fig. S1).

2.3. Clinical data

For the Edinburgh cases, baseline patient characteristics, treatment information and outcome data, collected prospectively as part of routine care, were retrieved from the Edinburgh Ovarian Cancer Database [6]. Data for LGSOC patients from the AMC and NKI was collected from patient file review. Disease-specific survival (DSS) was calculated from the date of confirmed LGSOC diagnosis. Progression-free survival (PFS) was recorded as the duration between the date of diagnosis to the date of first progression, as defined by abnormalities on radiological examinations, GCIG CA125 tumour marker progression, or death from LGSOC.

2.4. Whole exome sequencing, variant calling and analysis

Following DNA extraction from macro-dissected FFPE sections (Supplementary Methods), exome capture was performed using the Illumina TruSeq Exome Library Prep kit and WES was performed on the Illumina NextSeq 550 (Illumina, Inc., San Diego, CA, USA). Samples failed sequencing QC if mean on-target coverage was <30× ($n = 5$). Data were aligned to the GRCh38 human reference genome using bwa-0.7.17 [20], duplicates marked and base quality scores recalibrated with the GenomeAnalysisToolkit (GATK) v4 [21] within the bcbio 1.0.6 pipeline (Supplementary Methods).

2.5. Variant calling, microsatellite instability and pathway analysis

Variants were called using a majority vote system from three callers (VarDict [22], Mutect2 [23] and Freebayes [24]) and were filtered to remove common and likely non-functional variation (Supplementary Methods). Analysis of variants and oncogenic pathways was performed using the R package maftools (Supplementary Methods) [25]. SNV classes were defined with respect to genes linked to the MAPK pathway; cMAPKm = canonical MAPK mutations (*KRAS, BRAF, NRAS*), MAPK-assoc = Non canonical MAPK genes, MAPKwt = no MAPKm gene identified. Tumour mutational burden (TMB) was calculated as the number of mutations present following basic filtering to remove reads which failed technical quality control filters. TMB was stratified into quartiles across the cohort. Cancer gene sets were taken from gene lists defined by OncoKB, excluding "MSK Heme" and "Foundation One Heme" markers. Microsatellite instability (MSI) scores were defined using the MSI sensor 2 (https://github.com/niu-lab/msisensor2), which applies machine learning models to determine the MSI status for a distribution per microsatellite. MSI scores (number of MSI sites detected/ all valid sites with sufficient coverage) are then calculated with a threshold of 20% deemed to identify MSI cases.

2.6. Mutational signature analysis

The mutational signatures from the Catalogue of Somatic Mutations in Cancer (COSMIC) in which the mutational profiles are represented as the proportion of each substitution type (C > A, C > G, C > T, T > A, T > C, and T > G) and its trinucleotide context were employed. To investigate the mutational signatures present in our cohort we used the R package "deconstructSigs" using an iterative approach to calculate the combination of COSMIC SBS signatures and determine the COSMIC signatures (https://cancer.sanger.ac.uk/cosmic) that present in the tumour samples and the corresponding contribution of each signature toward the total mutational spectra (Supplementary Methods. [26,27].

2.7. Copy number analysis

Copy number (CN) analysis was performed using CopywriteR to determine relative CN estimates at 30Kb intervals across the genome, utilising aligned BAM files from the above bcbio workflow as an input (Supplementary Methods) [28]. Median relative CN across chromosome arms was calculated using hg38 chromosome band reference coordinates from University of California Santa Cruz (UCSC). A medium log2 CN ratio of −0.25 and 0.25 were used as threshold for loss and gain, respectively, yielding a sensitivity and specificity of 96.2% and 98.7% against a manually curated truthset of chromosome-arm-level CN events at chromosome 1–6 across the dataset (Supplementary Methods). Gene level CN counts were extracted from CopywriteR bins. CN changes across cancer genes were defined as change of log2 0.5. The hierarchical clustering of CN data represented in heatmap plots was carried out using Euclidean distance and Ward's linkage.

2.8. Statistical analysis and genomic model

Statistical analyses were performed using R version 4.0.3 or above. *t*-tests were used to compare continuous data. Survival analyses were performed using Cox proportional hazards regression analysis in the Survival package using a log rank to generate a *P* value for the difference between genomic classes. The genomic model was built through stepwise selection of cases based on the performance individual hazard ratios in the following order; i) TMB lower quartile samples [15/63 samples], ii) co-occurring Chr1p loss-1q gain [15/48 remaining samples], iii& iv) SNV class split by MAPK-associated/wt [14/33] remaining samples or cMAPKm [the remaining 19 samples].

3. Results

3.1. Cohort characteristics

118 LGSOC cases were identified (all WT1 positive with definitive stromal invasion). 41 cases were excluded for insufficient tumour material or cellularity; 3 failed DNA quality control. 74 cases underwent genomic characterisation by WES. 5 had insufficient sequencing coverage (<30×) and 6 cases harbouring *TP53* mutations were excluded as potential occult HGSOC, leaving 63 cases in the final LGSOC study cohort (Fig. S1). The median per-sample on-target coverage in the study cohort was 64× (range 32×-132×).

Clinical characteristics of the LGSOC study cohort are summarized in Table 1. The median follow-up time was 13.3 years. All patients underwent cytoreductive surgery. 15/63 cases received neoadjuvant chemotherapy prior to delayed primary surgery. The median disease-specific survival (DSS) was 12.9 years.

3.2. SNV subgroups of disease

Across the cohort, the total tumour mutational burden (TMB) varied widely (median 233, range 16–405). Microsatellite instability (MSI) scores were also low across all samples with no MSI-high cases noted. We identified canonical MAPK pathway mutations in 33 cases (52.4%) (cMAPKm subgroup): 24 *KRAS* (38.1%), 6 *BRAF* (9.5%) and 3 *NRAS* (4.8%), which occurred mutually exclusively (Fig. 1, Fig. S2 and S3). The majority of these events occurred at known mutational hotspots (83% of *KRAS* G12R/C/D/V, 100% *BRAF* V600E, 100% *NRAS* Q61R/K) (Fig. S4).

Pathway analysis of mutated genes revealed a further 17 cases (27.0%) with perturbation of the MAPK/RAS pathway by mutations across 14 other MAPK-associated genes defined by the TCGA PanCanAtlas [29] (MAPK-associated mutations) (Figs. 1 & S5). These events were largely mutually exclusive with one another, and with cMAPKm events (*KRAS*, *BRAF*, *NRAS* mutation) (Fig. 1). The total

Table 1
Clinical characteristics of low grade serous ovarian carcinoma cases.

		N	%
Cases	Total	63	
Age	Median	54 years	Range 19–66
Centre	Edinburgh Cancer Centre	31	49.2
	University of Amsterdam Academic Medical Centre	10	15.9
	Netherlands Cancer Institute	22	34.9
FIGO stage	I	9	14.5
	II	5	8.1
	III	39	62.9
	IV	9	14.5
	NA	1	–
RD following cytoreduction	Macroscopic RD	34	55.7
	No macroscopic RD	27	44.3
	Unknown RD status	2	–
Timing of surgery	Primary	48	76.2
	Post neoadjuvant chemotherapy	15	23.8
Diagnosis period	Pre-2000	10	15.9
	2000s	24	38.1
	2010 onward	29	46.0
Death details at last follow-up	Alive	25	39.7
	Died of OC	21	33.3
	Died of other causes	9	14.3
	Died, unknown cause	8	12.7
Outcome	5-year DSS	70.6%	95% CI 59.1–84.4
	Median follow-up	13.3 years	95% CI 6.36-NA

FIGO, International Federation of Gynecology and Obstetrics; NA, not available; RD, residual disease; OC, ovarian carcinoma; DSS, disease-specific survival.

frequency of MAPK/RAS pathway alteration was 50 of 63 cases (79.4%) (33 cMAPKm, 17 MAPK-associated mutations). The remaining MAPK wild-type (MAPKwt) cases contained a number of mutations over OncoKB defined cancer genes and demonstrated younger age at diagnosis compared to cMAPKm cases (median 47 vs 62 years, P value = 0.048) (Figs. 1, S6, S7a & Table S1). Overall TMB was also higher in both the cMAPKm and MAPKasociated groups (median TMB = 256 & 249 respectively) compared to MAPKwt samples (median TMB = 147. Fig. S7b).

NOTCH pathway perturbation was common across the cohort (21 cases, 33% of cases), occurring both in the context of MAPK/RAS pathway alteration and in MAPKwt cases (34% and 31%, respectively) (Figs. 1 & S5). Recurrent gene targets of mutation beyond MAPK pathway components included *ATM* (5 cases, 7.9%), *USP9X* (5 cases, 7.9%) and *EIF1AX* (3 cases, 4.8%) (Figs. S1 & Fig. S3).

3.3. Analysis of mutational signatures in low grade serous ovarian carcinoma

To explore the mutational processes in LGSOC, mutational signatures were characterised based on the 96 possible mutation types in the total datasets. Signature SBS1, which is associated with ageing, was most evident across the cohort (present in 88.9% of samples, Fig. 2A, B & S8). Signatures SBS24 (84.1%), SBS37 (61.9%) and SBS48 (33.3%) were also detected but are poorly annotated in terms of functional relationships. By contrast SBS10b was detected in around a quarter of the cohort (23.8%) and was associated with hypermutated tumours, reflected in the elevated TMB in tumours with this signature (median TMB SBS10b detected = 256, SBS10b negative = 172, Figs. 2B & S9). Focussed analysis of the genes mutated in samples displaying a SBS10b signature identified elevated levels of *KRAS* mutation in samples with a SBS10b signature (60% SBS10b enriched vs 31% SBS10b negative).

3.4. Copy number alterations occur both over chromosomal arms and at cancer genes

At the arm level the most common events were loss of chr1p (42.9%, 27 cases), 22q (31.7%, 20 cases), 9p (23.8%, 15 cases), Xq (23.8%, 15 cases) and 18p (22.2%, 14 cases), and gain of 1q (36.5%, 23 cases), 19p (34.9%, 22 cases), and 8q (19.0%, 12 cases). Loss of chr1p and gain of chr1q significantly co-occurred (20 co-occurrences, $P < 0.0001$, Fig. 3A, B & S10). Chromosome arm CN events were evenly distributed between the genomic subgroups; highest in number in the cMAPKm and MAPK-associated SNV classes compared to MAPKwt cases. (Fig. 3C, S11 & Table S2).

CN changes over established cancer genes identifies two clusters of CN events (CN high and CN low groups. Figs. S12 & S13). Across these genes CN gain of *KMT2D* and loss of *JUN* are the most frequently altered events (Fig. 3D). In addition, we report 22 cases with loss at *CDKN2B* locus (34.9%), 8 cases with loss at *CDKN2A* locus (12.7%) and 3 cases demonstrated loss of *USP9X*, (4.8%; none of which co-occurred with USP9X mutations) (Log2 0.5 fold change (FC), Fig. 3D).

3.5. Features associated with clinical outcomes

Following adjustment for tumour stage and residual disease after cytoreduction, both cMAPKm and MAPK associated tumours displayed favourable DSS hazard ratios with respect to MAPKwt as a reference (cMAPKm HR: 0.32, 95% CI 0.10–0.99, Pval 0.048; MAPKassoc HR: 0.61, 95% CI 0.17–2.21, Pval 0.44. Table 2 & Fig. S14). LGSOC cases with TMB scores in the lower quartile experienced significantly shorter DSS (HR for lower quartile TMB 6.43, 95% CI 2.24–18.47, Pval <0.001. Table 2). Similarly cases which exhibited concurrent chr1p CN loss & chr1q CN gain ("Chr1pq CN") experienced significantly shorter DSS (HR for Chr1pq CN 3.29, 95% CI 1.31–8.28, Pval 0.011. Table 2). Numbers of total gene level CN gain and loss events were not related to overall survival differences (data not shown).

Taken together, we constructed a stepwise genomic classification based on the effect size of the hazard ratios for DSS. In doing so, we identified four genomic classes: i) TMB low samples, ii) co-occurring Chr1pq CN samples, iii) MAPKwt & MAPK associated SNVs [TMB high/Chr1pq CN normal] & iv) cMAPKm SNVs [TMB high/Chr1pq CN normal] (Fig. 4A & Table 3). Group i (24% cohort), characterised by low TMB and low gene level CN counts, is relatively enriched in MAPKwt tumours (47% of the group) and displays poor 5 year DSS (46%) and PFS (40%) (Figs. 4B, S15 & Table 3). Group ii cases (24%) are typically older at diagnosis and characterised by concurrent chromosome 1p CN loss with 1q CN gain as well as higher TMB and gene level CN. Similar to group i, these cases display poor 5 year DSS (55%) and PFS (42%)(Figs. 4B, S15 & Table 3). The remaining tumours (high TMB and Chr1pq CN normal) are split by MAPK mutational status into groups iii) MAPKwt and non-canonical MAPK tumours or iv) canonical MAPK mutations. Cases in group iii display the youngest age at diagnosis, are TMB high and gene level CN low with favourable 5 year DSS (79%) and PFA (64%)(Figs. 4B, S15 & Table 3). Cases in group iv harbour mutations in either *KRAS*, *BRAF* or *NRAS*, are enriched for the COSMIC signature SBS10b, are TMB high and gene level low and display remarkable 5 year DSS (100%) and PFS (75%. Figs. 4B, S15 & Table 3).

4. Discussion

LGSOC affects younger women than HGSOC and demonstrates greater levels of intrinsic chemoresistance. Although survival is better than HGSOC, patients frequently relapse resulting in morbidity from often ineffective therapies and premature mortality. Additional treatment options and an improved understanding of LGSOC biology are therefore required to improve patient outcomes.

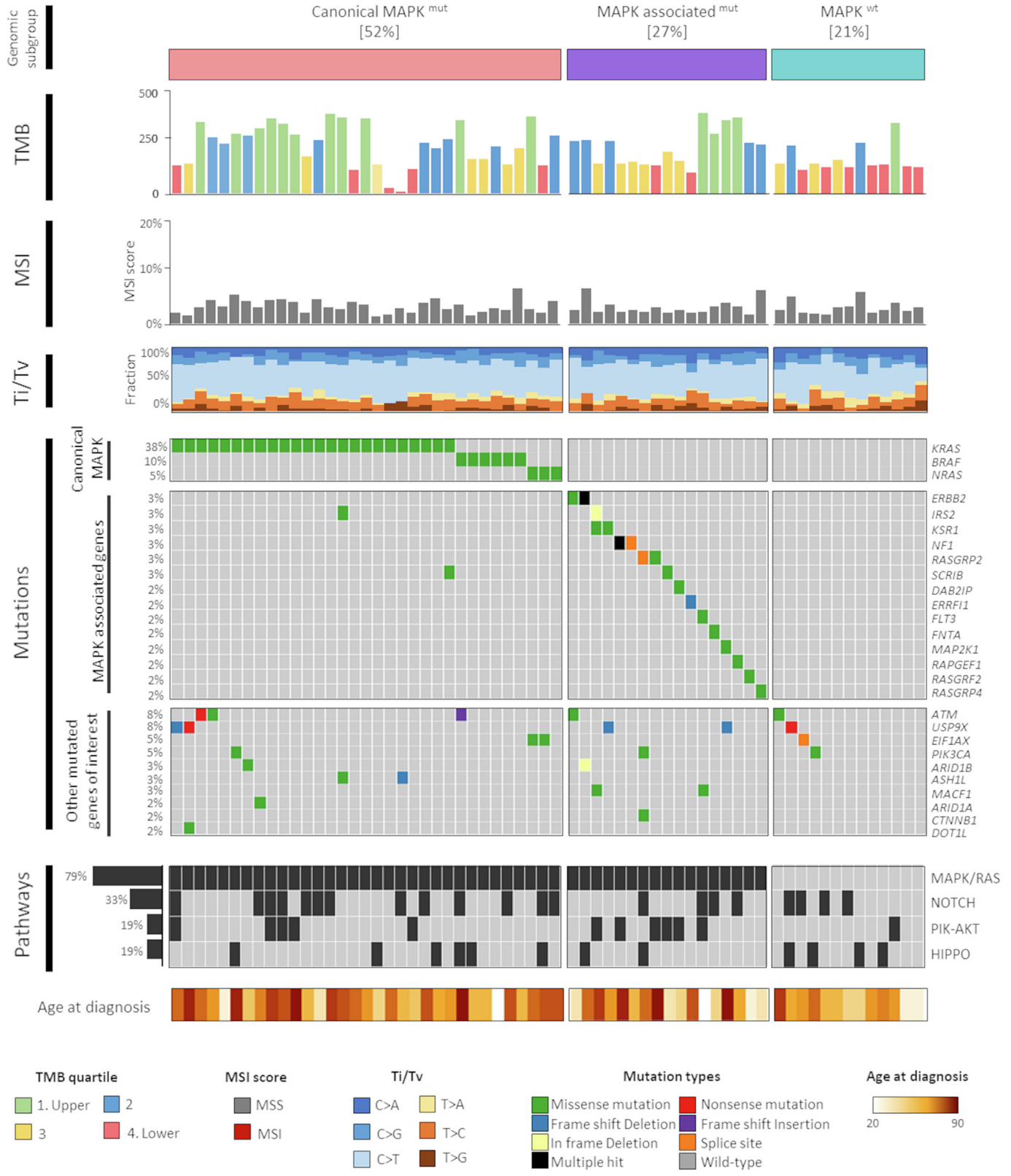

Fig. 1. Molecular landscape of low grade serous ovarian carcinoma. TMB, tumour mutational burden; MSI, microsatellite instable; MSS, microsatellite stable Ti, transitions; Tv, transversions; SNV, single nucleotide variant; cMAPKm, canonical MAPK pathway mutation (*KRAS*/*BRAF*/*NRAS*); MAPK-associated, MAPK-associated mutation; MAPKwt, MAPK pathway wild-type.

To date, few studies have carried out comprehensive genomic characterisation of robustly curated LGSOC cohorts. Where molecular profiling studies have been performed, these have largely been limited to panel based sequencing of selected genes [7,9,16], or have included only a small number of patient samples [11,17]. Here we report comprehensive genomic profiling by WES, analysing this genomic data to better

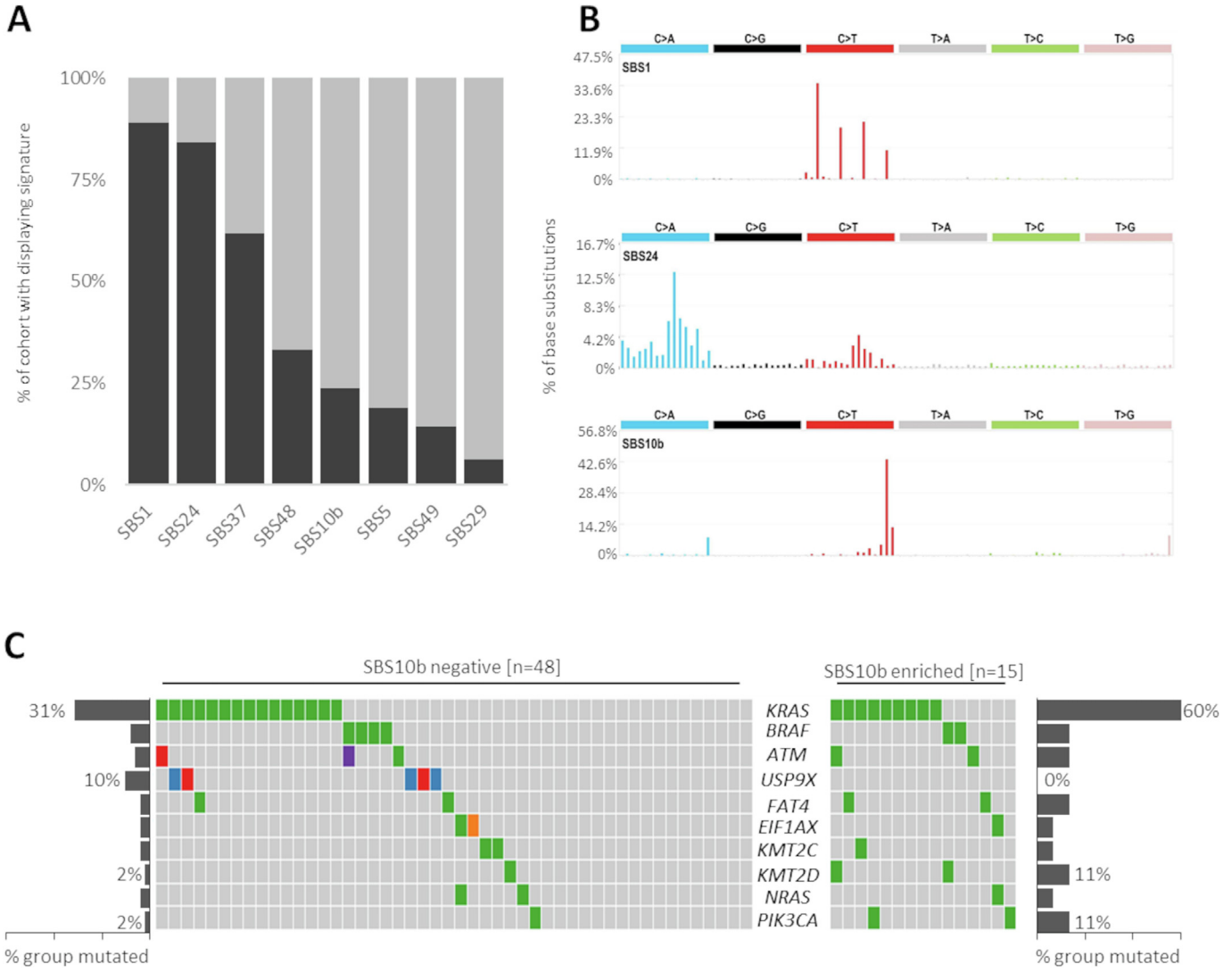

Fig. 2. Mutational signatures in LGSOC. (A) Plot of the most frequent COSMIC SBS signatures identified across the whole cohort (%). (B). Mutational profiles for SBS1, SBS24 and SBS10b using conventional 96 mutation type classifications (C). Oncoplot of the top 10 most frequently mutated cancer genes split by SBS10b signature status. Genes displaying >10% difference in frequency between the two groups are displayed.

understand both SNV, mutational signatures and CN events in a sizeable LGSOC cohort.

Across our cohort, 52% of cases harboured SNV mutations in the canonical MAPK pathway components *KRAS*, *BRAF* and *NRAS*; similar to the frequency reported in other studies [7,18,19]. In addition, we also identified a further 27% of cases (MAPK-associated group) demonstrating mutational disruption of genes reportedly involved in the MAPK pathway [29]. Previous studies have alluded to a proportion of LGSOC patients with mutations in MAPK pathway components beyond canonical *KRAS*, *BRAF* and *NRAS* mutations, such as inactivating *NF1* mutations [7,18,19]. However, the phenotype of these cases is poorly characterised. Given that two late phase trials of MEK inhibitors have suggested improved response rate to MEK inhibition (trametinib, binimetinib) in cases with canonical MAPK mutations [14,15], the expansion to also consider MAPK associated cases may identify additional patients more likely to respond to MEK inhibitors.

Consistent with recent reports, the remaining MAPKwt cases (21%) represented patients with younger age at diagnosis (median 47 years) [18,19]. This suggests that previous observations of poorer survival in younger LGSOC patients may be underpinned by differences in disease biology between younger and older patients [2]. Focussed analysis of these MAPKwt cases discovered a series of potential driver events at oncogenes and tumour suppressor genes but these events rarely occurred over recurrent sets of genes.

Outside gene level SNV events, we note a spread in overall mutational burdens within the cohort. Similar to previous studies in ovarian cancer we report that LGSOC cases with lower TMB counts displayed significantly reduced disease-specific survival than those with higher TMB [30]. Tumour mutations are considered to be a vital process in the development of anti-cancer immunity, and it is well recognised that heavily mutated tumours harbour more neoantigens, which allow them to become a target of stimulated immune cells [31]. As such, TMB high LGSOC patients may represent a group suitable for checkpoint inhibition based therapeutic intervention.

In addition to SNV events across a number of genes, we detect the presence of a number of mutational signatures identified by analysis of the trinucleotide changes across the entire exome. Similar to previous reports in HGSOC, we detect a strong enrichment of signature SBS1 which has been reported to correlate with age of the individual as well as possible deamination of 5-methylcytosine bases [32]. We also detect a number of poorly defined signatures as well as SBS10b, a signature associated with elevated TMB. Interestingly, cases enriched in this signature demonstrated significantly elevated DSS compared to those without, highlighting a potential biomarker for good prognosis in LGSOC patients.

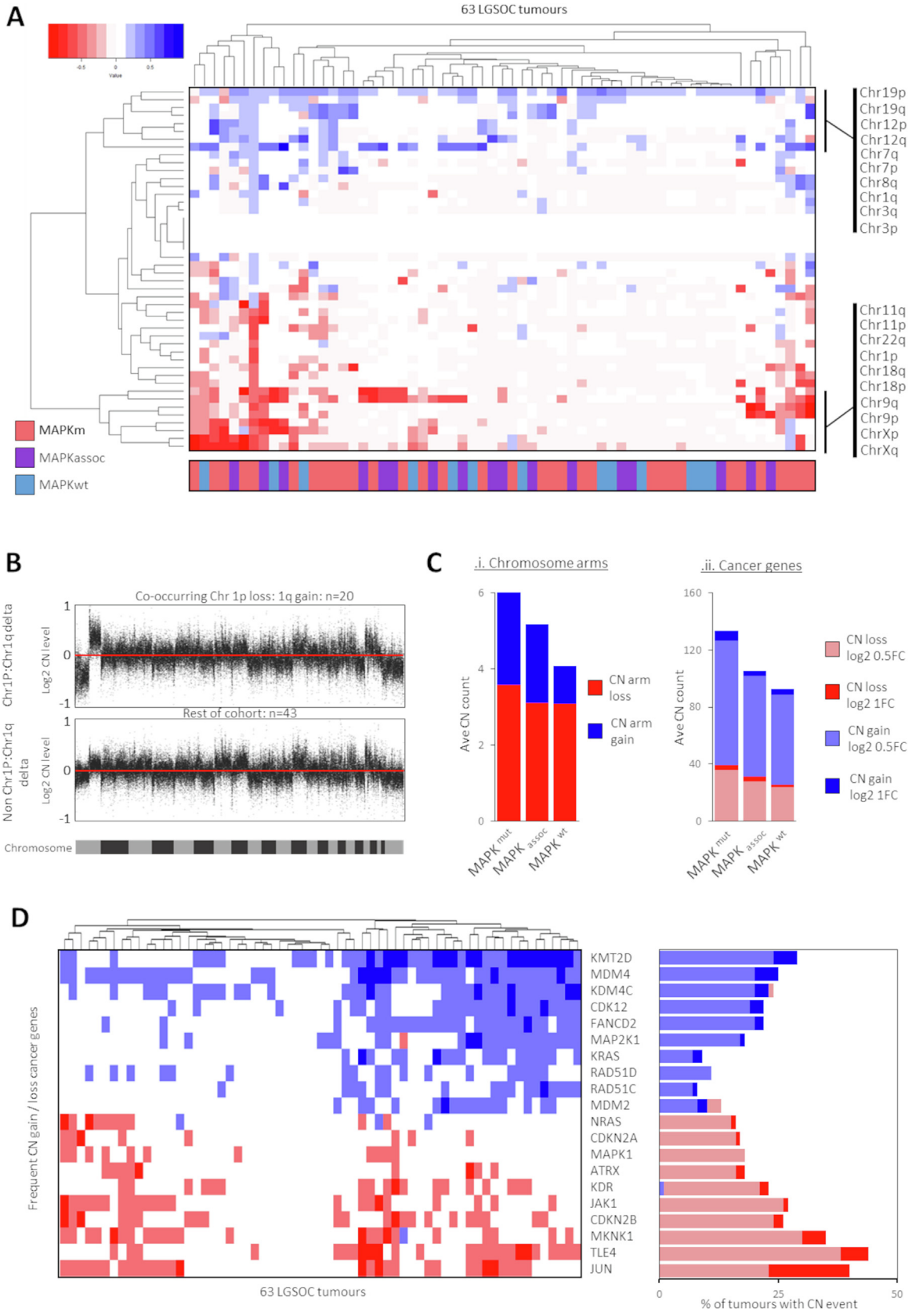

Fig. 3. CN events in LGSOC. (A). Heatmap of chromosome arm CN changes. Average Log2 fold change (FC) scores across chromosome arms are plotted. SNV classification is plotted below. cMAPKm, canonical MAPK pathway mutation (*KRAS*/*BRAF*/*NRAS*); MAPK-associated, MAPK-associated mutation; MAPKwt, MAPK pathway wild-type. (B) Average profiles of CN windows across co-occurring Chr1p loss: Chr1q gain tumours (upper plot) and non-co-occurring Chr1p loss: Chr1q tumours. (C) (i) Plot of chromosome arm loss (red) and gain (blue) events, split by SNV class. (ii). Plot of cancer gene CN count of loss (pink & red) and gain (light blue and dark blue), split by SNV class. (D). Heatmap of frequent cancer gene CN changes. Colours correspond to those in Fig. 2C ii.

Table 2
Analysis of disease-specific survival split by either SNV class, TMB or Chr1pq CN status following adjustment for tumour stage and residual disease. HR, hazard ratio; P, P value; cMAPKm, canonical MAPK mutant; MAPK-assoc, MAPK-associated mutation; MAPKwt, MAPKwild-type. Sig, COSMIC signature; TMB, tumour mutational burden; Q; quartile (1 high, 4 low); CN, copy number; DSS, disease-specific survival; PFS, progression free survival.

		HR	95% confidence interval	P
SNV class	cMAPKm	0.32	0.10–0.99	0.048
	MAPK-assoc	0.61	0.17–2.21	0.44
	MAPKwt	Ref	Ref	Ref
TMB	TMB Q4 (low)	6.43	2.24–18.47	<0.001
	TMB Q1–3	Ref	Ref	Ref
Chr1pq CN	Present	3.29	1.31–8.28	0.011
	Absent	Ref	Ref	Ref

Aside from SNV genomic changes, we also observe copy number loss of *CDKN2B* (34.9%), *CDK2NA* (12.7%) and *USP9X* (4.8%), which are consistent with published data [7]. As previous work has shown that *CDKN2A* aberrations are enriched in LGSOC cases with a shorter overall survival, targeting *CDKN2A* may represent a promising strategy for therapeutic intervention in these patients [7]. At the chromosomal level, we detect frequent loss of chr1p (42.9%), 22q (31.7%), 9p (23.8%), Xq (23.8%) and 18p (22.2%), and gain of 1q (36.5%), 19p (34.9%), and 8q (19.0%). Across the cohort, cases with co-occurring Chr1p CN loss: Chr1q CN gain demonstrated strikingly significantly poorer DSS as well as shortened progression free survival durations. Employment of whole genome sequencing approaches to better characterise CN changes as well as larger structural variation changes such as translocations, inversion, large deletions and amplifications with matched normal datasets will greatly advance our understanding of more complex genomic events in LGSOC, including those at Chr1, which to date are lacking.

Stratification based on the genomic analyses results in four groups with differing disease specific outcomes. Patients in groups i (low TMB) and ii (co-occurring Chr1pq CN events) represent the poorest DSS and PFS groups, with around half of these patients demonstrating progression and ultimately dying of their disease within 5 years. Cases with higher TMB and demonstrating non-co-occurring Chr1pq CN events perform favourably in terms of both DSS and PFS. These groups can be further stratified by MAPK mutational status, with cases harbouring canonical MAPK mutations demonstrating superior DSS and PFS compared to those with MAPK-associated mutations or lacking mutations in the MAPK pathway.

Both the GOG281/LOGS and MILO studies suggested that the response rates to MEK inhibition were greater in patients with canonical MAPK defects [14,15]. However, to date, no assessment of relative MEK inhibitor efficacy has been made with regard to MAPK pathway mutations beyond *KRAS*/*BRAF*/*NRAS*; future work should seek to quantify efficacy within these patients, and should determine whether patients with no identifiable genomic MAPK defect (MAPKwt) derive any benefit at all from MEK inhibition. The exact location of activating mutation within the MAPK pathway may also have implications for the choice of partner in MEK inhibitor combination strategies seeking to abrogate resistance mediated through crosstalk between intracellular signal transduction pathways (e.g. combinations with the FAK inhibitor dabrafenib).

Beyond the MAPK pathway, analysis of genomic events from our WES data suggest perturbation of the NOTCH signalling pathway is common, affecting a notable proportion of cases both with and without MAPK defects (34% and 31%, respectively). NOTCH pathway inhibition may therefore represent a novel therapeutic strategy for LGSOC patients with intrinsic or acquired resistance to chemotherapy and/or MEK inhibitors. In this regard, a recent study suggested that NOTCH signalling limits the response of LGSOC to MEK inhibition [33].

We report the first investigation using WES in a sizeable unselected LGSOC cohort ($n = 63$) with detailed SNV, mutational signature and copy number analysis as well as clinical annotation and extensive follow-up. The use of immunohistochemistry for WT1 and p53 status to robustly curate our cohort, represents a major strength of this

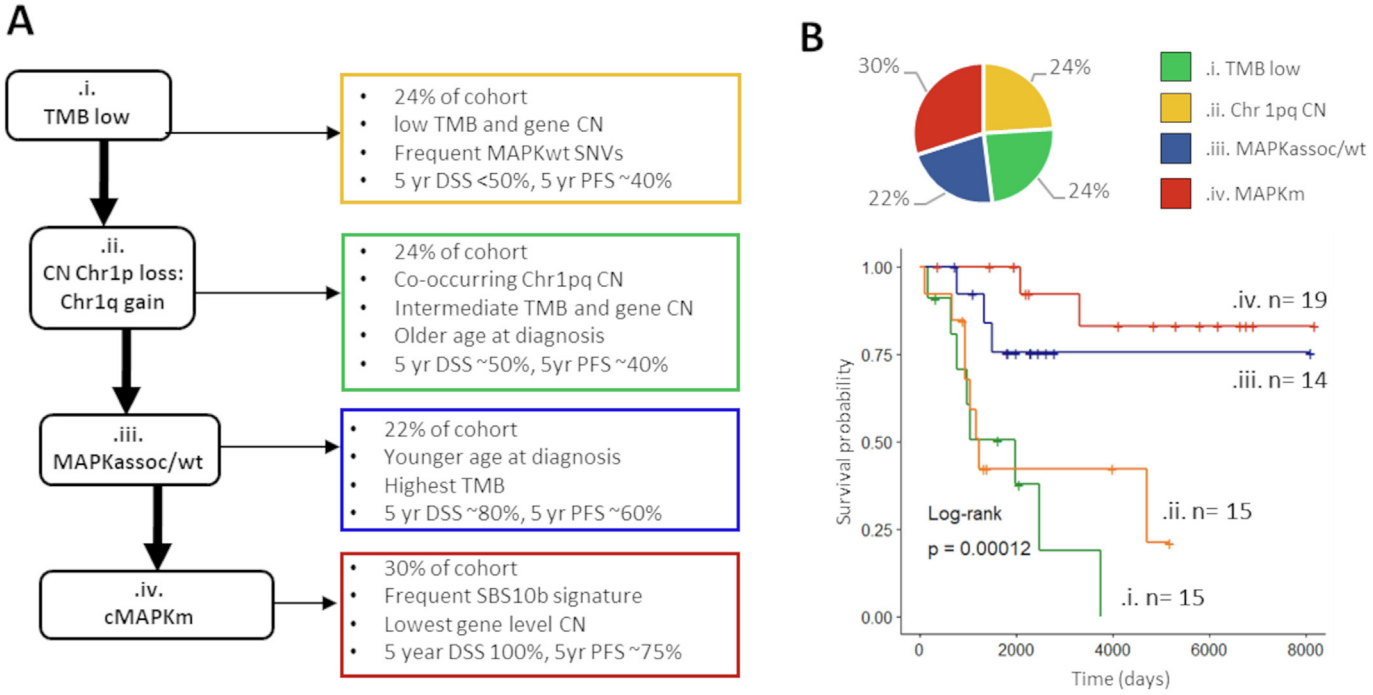

Fig. 4. Impact of low grade serous ovarian carcinoma features on disease-specific survival (DSS). (A) Summary of hierarchical genomic classification. (B) DSS of patients split by genomic class. Time in days, HR: hazard ratio, 95% CI: 95% confidence interval. cMAPKm, canonical MAPK pathway mutation (*KRAS*/*BRAF*/*NRAS*); MAPK-assoc, MAPK-associated mutation; MAPKwt, MAPK pathway wild-type. Number of cases per group are shown on the plot.

Table 3
Characteristics of low grade serous ovarian carcinoma cases, spit by genomic classes. cMAPKm, canonical MAPK mutant; MAPK-assoc, MAPK-associated mutation; MAPKwt, MAPKwild-type. Sig, COSMIC signature; TMB, tumour mutational burden; CN, copy number; DSS, disease-specific survival; PFS, progression free survival.

		.i. TMB low	.ii. CN Chr1pq	.iii. MAPKwt/ MAPKassoc	.iv. cMAPKm
LGSOC Molecular Class	% group [n=]	24% [15]	24% [15]	22% [14]	30% [19]
Age	Median age at diagnosis (years)	54	63	50	53
	cMAPKm	40% [6/15]	53% [8/15]	0% [0/14]	100% [19/19]
SNV class	MAPK-assoc	13% [2/15]	33% [5/15]	71% [10/14]	0% [0/19]
	MAPKwt	47% [7/15]	13% [2/15]	29% [4/14]	0% [0/19]
SBS10 sig.	% group	7% [1/15]	7% [1/15]	21% [3/14]	53% [10/19]
TMB	Median mutations	134	256	260	254
CN count	Median cancer gene CN gain & loss events	74	168	77	68
	5 year DSS% where evaluable	46%	55%	79%	100%
Outcome	Anytime DSS % where evaluable	38%	27%	79%	88%
	5 year PFS survival % where evaluable	40%	42%	64%	75%

work. However, while our dataset represents one of the largest comprehensively characterised LGSOC collections reported to date, the overall cohort size remains modest due to the rarity of LGSOC. Confirmatory studies characterising the behaviour of the MAPK-associated SNV subgroup, and of the four genomic classes are therefore required. The data presented here paints a more detailed molecular portrait of LGSOC as a unique disease entity.

5. Conclusion

Collectively, our data identify a subgroup of LGSOC with mutations in MAPK pathway components beyond *KRAS*, *BRAF* and *NRAS*. Recurrent copy number events appear to occur independent of genomic subgroup. We identify genomic parameters linked with clinical outcome including SNV, TMB, mutational signatures and copy number events resulting in the generation of distinct genomic subclasses. In addition, genomic perturbation of the NOTCH pathway is common across LGSOC, and NOTCH pathway inhibitors may therefore warrant investigation as a potential therapeutic strategy to address the current unmet clinical need in LGSOC patients.

Author contributions

JPT: formal analysis, investigation, methodology, visualisation, data analysis, writing – original draft. RLH: formal analysis, investigation, methodology, visualisation, writing – original draft. JvB: investigation, methodology, conceptualisation, manuscript writing – review and editing. NI: investigation, methodology, conceptualisation; manuscript writing – review and editing. MC: data curation, project administration, writing – review and editing. KvdV: investigation, methodology, writing – review and editing. FD: investigation, methodology, writing – review and editing. AMM: investigation, methodology, writing – review and editing. CB: data curation, writing – review and editing. TR: data curation, investigation, writing – review and editing. IC: investigation, writing – review and editing. PD: investigation, writing – review and editing. MvG: investigation, methodology, writing – review and editing. HC: resources, writing – review and editing. RN: resources, writing – review and editing. FN: resources, writing – review and editing. CL: conceptualisation, supervision, methodology, writing – review and editing. CSH: investigation, supervision, methodology, writing – review and editing. CG: conceptualisation, supervision, methodology, writing – review and editing.

Ethics and approval to participate

Ethical approval was obtained from the Lothian Human Annotated Bioresource (reference 15/ES/0094-SR925), NKI-AVL Translational Research Board (reference CFMPB284), and University of Amsterdam AMC Biobank Assessment Committee (reference 2016_070#A201641). All participants provided written informed consent or had consent waived by the ethics committee due to the retrospective nature of the study.

Consent for publication

Not applicable.

Competing interests

RLH: consultancy fees from GlaxoSmithKline. FN: non-personal interests in AstraZeneca and Tesaro. CG: grants from AstraZeneca, MSD, BMS, Clovis, Novartis, BerGenBio, Medannexin and Artios; personal fees from AstraZeneca, MSD, GSK, Tesaro, Clovis, Roche, Foundation One, Chugai, Takeda, Sierra Oncology, Takeda and Cor2Ed outside the submitted work; patents PCT/US2012/040805 issued, PCT/GB2013/053202 pending, 1409479.1 pending, 1409476.7 pending, and 1409478.3 pending. CSH: none.All other authors declare no conflicts of interest.

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Data availability

The genomic data presented in this manuscript is available upon reasonable request via the European Genome-Phenome Archive (EGA: https://ega-archive.org/), under accession number EGAS00001006859.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2023.04.011.

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