

NIH Public Access Author Manuscript

Published in final edited form as:

J Pathol. 2012 February ; 226(3): 413-420. doi:10.1002/path.3967.

Low-grade serous carcinomas of the ovary contain very few point mutations

Siân Jones^{1,#}, Tian-Li Wang^{2,#}, Robert J Kurman², Kentaro Nakayama³, Victor E Velculescu¹, Bert Vogelstein¹, Kenneth W Kinzler¹, Nickolas Papadopoulos¹, and le-Ming Shih^{2,*}

¹Ludwig Center for Cancer Genetics and Therapeutics and Howard Hughes Medical Institute, Johns Hopkins Kimmel Cancer Center, Baltimore, MD 21231, USA

²Departments of Pathology, Oncology, Gynaecology and Obstetrics, Johns Hopkins Medical Institutions, Baltimore, MD 21231, USA

³Department of Gynaecology and Obstetrics, Shimane University, Izumo, Japan

Abstract

It has been well established that ovarian low-grade and high-grade serous carcinomas are fundamentally different types of tumours. While the molecular genetic features of ovarian highgrade serous carcinomas are now well known, the pathogenesis of low-grade serous carcinomas, apart from the recognition of frequent somatic mutations involving KRAS and BRAF, is largely unknown. In order to comprehensively analyse somatic mutations in low-grade serous carcinomas, we applied exome sequencing to the DNA of eight samples of affinity-purified, low-grade, serous carcinomas. A remarkably small number of mutations were identified in seven of these tumours: a total of 70 somatic mutations in 64 genes. The eighth case displayed mixed serous and endometrioid features and a mutator phenotype with 783 somatic mutations, including a nonsense mutation in the mismatch repair gene, MSH2. We validated representative mutations in an additional nine low-grade serous carcinomas and 10 serous borderline tumours, the precursors of ovarian low-grade, serous carcinomas. Overall, the genes showing the most frequent mutations were BRAF and KRAS, occurring in 10 (38%) and 5 (19%) of 27 low-grade tumours, respectively. Except for a single case with a *PIK3CA* mutation, other mutations identified in the discovery set were not detected in the validation set of specimens. Our mutational analysis demonstrates that point mutations are much less common in low-grade serous tumours of the ovary than in other adult tumours, a finding with interesting scientific and clinical implications.

Keywords

ovarian cancer; exome sequencing; BRAF; KRAS; somatic mutations

Copyright © 2012 Pathological Society of Great Britain and Ireland. Published by John Wiley & Sons, Ltd.

Correspondence to: Ie-Ming Shih, Johns Hopkins Medical Institutions, 1550 Orleans Street, CRB-2 305 Baltimore, MD 21231, USA. ishih@jhmi.edu. #Both authors contributed equally.

Conflicts of interest. Under agreements between the Johns Hopkins University, Genzyme, Exact Sciences, Inostics, Qiagen, Invitrogen and Personal Genome Diagnostics, KWK, VEV, BV and NP are entitled to a share of the royalties received by the University on sales of products related to genes and technologies described in this manuscript. KWK, VEV, BV and NP are co-founders of Inostics and Personal Genome Diagnostics, are members of their Scientific Advisory Boards and own Inostics and Personal Genome Diagnostics stock, which is subject to certain restrictions under Johns Hopkins University policy.

Author contributions

SJ, KWK, VEV, BV and NP designed the study and analysed sequencing data; T-LW, RJK, KN and I-MS designed the study, collected and purified samples, performed clinical correlation and prepared the manuscript.

Introduction

Ovarian cancer is not the most common gynaecological malignancy but it is the most lethal, accounting for nearly 140 200 deaths worldwide [1]. It is a heterogeneous disease composed of several distinctly different cell types. Among them, low-grade serous carcinoma (LGSC) constitutes a relatively unusual but distinctive type that tends to affect younger women more than high-grade serous carcinoma, the most common histopathological type [2–5]. In the past, it was thought that LGSCs progressed to high-grade serous carcinomas but it is now recognized that LGSCs and high-grade serous carcinomas generally develop along distinctly different molecular pathways [6]. As compared to high-grade ovarian serous carcinomas, LGSCs proliferate slowly and are clinically less aggressive. Although LGSCs have been termed 'low-grade', patients with this disease not infrequently develop recurrences and experience a protracted clinical course, requiring multiple surgical interventions, before succumbing to the disease, and the 10 year survival is < 50% [7].

LGSCs may develop from serous cystadenomas, which can progress to a serous borderline tumour (SBT), also known as atypical proliferative serous tumours (APSTs), non-invasive (micropapillary) LGSCs, then to invasive LGSC [8–11]. Hemizygous ch1p36 deletion and ch9p21 homozygous or hemizygous deletions may play an important role in this pathway, because deletions of both chromosomal regions are more frequent in LGSCs than in SBTs (APSTs) [12]. Mutational analyses of candidate genes have shown that both LGSC (non-invasive and invasive) and SBTs (APSTs) harbour somatic mutations in *KRAS* and *BRAF* in approximately half of the cases [13–16]. The aim of the present study was to comprehensively analyse the mutation profile of ovarian LGSCs by exome sequencing. Genome-wide mutation profiles of ovarian high-grade serous carcinomas [17] and clear cell carcinomas have recently been reported [18, 19]. The results reported here complement the other studies and highlight genetic differences among the different types of ovarian carcinomas.

Materials and methods

Tissue specimens

We analysed the exomes of eight LGSCs in a 'discovery set' and all specimens were reviewed by two gynaecological pathologists (RJK and IMS), using the criteria previously detailed [33], and microscopically all tumours exhibited a low-grade nuclear feature. These consisted of six carcinomas exhibiting morphologically pure serous differentiation and two with serous plus either clear cell or endometrioid features, respectively. Among them, four cases were recurrent and four were primary tumours. Tumour cells from LGSCs were isolated from fresh surgical specimens by collagenase I digestion, followed by epithelial cell enrichment using Dynal beads coated with Epi-CAM antibodies, as previously described [18]. The affinity-purified tumour cells were cultured in glass chamber slides overnight, fixed using 3% paraformaldehyde at room temperature for 10 min, and washed three times in phosphate-buffered solution containing 0.2% Triton X-100 (Sigma). The cells were then incubated with an antibody reacting to cytokeratin 17 (DAKO, Carpentaria, CA, USA) at a dilution of 1:20 at room temperature for 1 h. The immunoreactivity was visualized using the DAKO EnVision (HRP) + system (DAKO), following the manufacturer's protocol. Haematoxylin was used as a nuclear counterstain. To determine the purity of tumour cells after isolation, we determined the percentage of positive cells, as defined by intense nuclear staining, by randomly counting at least 100 cells at ×20 magnification.

Genomic DNA preparation

Genomic DNA (from both tumour and normal tissues) was purified using Qiagen DNA blood kits, following the manufacturer's protocol. The DNA from the normal counterpart came from peripheral blood lymphocytes in three cases, from stromal cells of normal fallopian tube in four cases and from normal liver in one case. In the validation set, there were nine LGSCs (four recurrent and five primary tumours) and 11 SBTs (APSTs), the precursor of LGSCs. The criteria used to select SBT cases were based on the following morphological features: extensive epithelial stratification; tufting; and detachment of individual cells and small cell clusters besides hierarchical branching, with successively smaller papillae emanating from the larger, more centrally located papillae. Of note, to warrant a diagnosis of SBT, we included only those cases with stratification and budding in at least 10% of the tumour. Among the 11 SBTs (APSTs), there were two containing a noninvasive (in situ) LGSC component. To purify genomic DNA from SBTs (APSTs), we incubated fresh tissue fragments of SBTs (APSTs) with 0.5% trypsin and EDTA at 37 °C for 20 min with agitation. The tumour cells on the surface of papillae were gently scraped off, the epithelial cells were seeded in tissue culture flasks overnight and blood cells were removed after several washes before the attached epithelial cells were harvested for DNA purification, as described above. Tissue acquisition was approved by the Institutional Research Board.

Exome sequencing and analysis

Preparation of Illumina genomic DNA libraries and exome and targeted subgenomic DNA capture were performed as described [18]. Briefly, after capture of the coding sequences of the targeted genes using a SureSelect Enrichment System, the DNA was sequenced using an Illumina GAIIx Genome Analyser. Paired-end sequencing, resulting in 75 bases from each end of the fragments, was employed. The tags were aligned to the human genome reference sequence (hg18) using the Eland algorithm of CASAVA 1.6 software (Illumina). The chastity filter of the Base-Call software of Illumina was used to select sequence reads for subsequent analysis. The ELANDv2 algorithm of CASAVA 1.6 software (Illumina) was then applied to identify point mutations and small insertions and deletions. Known polymorphisms recorded in dbSNP were removed from the analysis. A mismatched base was considered to be a putative somatic mutation only when: (a) it had a QS score > 20; (b) it was identified in more than three distinct tags; (c) the percentage of mutant tags in the tumour was > 10%; (d) it was not found in > 2% of the tags in the matched normal sample; (e) it was a non-synonymous change; (f) there were no repeated regions homologous to the mutated sequence (as determined by BLAT analysis); and (g) the normal distinct Phred coverage was > 10.

Sanger sequencing for validation

Putative mutations identified by exome sequencing were validated by Sanger sequencing. Those that were detected through Sanger sequencing were considered true somatic mutations. Additional LGSCs and SBTs (APSTs) were sequenced using conventional Sanger methods to identify the prevalence of mutations in *KRAS, BRAF, PIK3CA, ARID1A, TSPAN11, STYK1, RNF214, DDC* and *SMARCA4.* PCR and Sanger sequencing were performed as previously described [18, 20]. To confirm mutations based on exome sequencing data, we used the primers specific for the exon the potential mutation was found. To analyse the mutation status of the candidate genes in additional cases, we screened the entire coding region.

Results

To maximize sensitivity for detecting somatic mutations, we analysed neoplastic cells enriched by immunopurification from fresh specimens. Immunostaining of the purified epithelial cells confirmed that > 90% of them were epithelial. The cells isolated from eight patients were used to determine the sequences of the approximately 18 000 protein-encoding genes listed in the RefSeq database (see Supporting information, Table S1). DNA from the normal tissues of each of these eight patients was also used for exomic sequencing. Using the Illumina GAIIx platform, the average coverage of each nucleotide in the targeted regions was 75-fold and 92.1% of those bases were represented in at least 10 reads.

OV207 displayed more than 50-fold more mutations than the other tumours and this tumour was found to harbour a somatic nonsense mutation in MSH2 (g.chr2 : 474969666C > T; c. 970C > T; p.324Q > X). Table S2 (see Supporting information) lists the 783 genes with somatic mutations found in OV207. Accordingly, OV207 was considered to be mismatchrepair deficient and was not considered for further analysis. Using stringent criteria for analysing the data from the other seven tumours [18], we were able to detect 85 somatic mutations (all listed in Table 1) and, among them, 70 somatic mutations in 65 genes could be confirmed by Sanger sequencing, and thus the false-positive rate of exome sequencing in this study was 17.6%. The validated somatic mutations per tumour averaged 10 for the 7 cases (range 0-24; Table 1). The six morphologically pure LGSCs (OV202, OV203, OV204, OV205, 0V206 and OV209) harboured \$\angle 0\$ mutations, and one low-grade serous tumour (OV208) showing focal clear cell feature had 24 mutations. Thus, the somatic nonsynonymous and splice site mutations/tumour was 7.5 in the 6 morphologically pure LGSCs. OV207, which was presumably mismatch-repair-deficient, exhibited mixed serous and endometrioid features. One tumour, OV202, did not harbour any detectable point mutations and its clinico-pathological features were similar to those of other low-grade serous neoplasms. The mutations detected in the pure LGSCs included BRAF, KRAS, STYK1, TSPAN11, RNF214, CCDC76 and SPATA5.

Using Sanger sequencing, we determined the mutation status of *KRAS*, *BRAF*, *PIK3CA*, *ARID1A*, *TSPAN11*, *STYK1*, *RNF214*, *DDC* and *SMARCA4* in affinity-purified tumour samples from additional cases, including nine LGSCs and 10 SBTs (APSTs). *PIK3CA*, *ARID1A*, *TSPAN11*, *RNF214* and *DDC* were selected for further study, based on their mutations being found in more than one case. *KRAS* and *BRAF* were analysed because mutations in both genes have been reported in LGSCs. *SMARCA4* was selected, despite being mutated in only the sample OV207, because it encodes Brg1, a component of the SWI/SNF chromatin remodelling complex, by interacting with ARID1A. *STYK1* was also studied because it encodes a receptor protein tyrosine kinase that may be of biological and translational significance. We found that besides *BRAF*, *KRAS* and *PIK3CA*none of the other genes showed somatic mutations in any of the samples in the validation set (Table 2).

Combining specimens from both the discovery and validation sets revealed that among 15 morphologically pure LGSCs, four contained *KRAS* mutations, three contained *BRAF* mutations and one had a *PIK3CA* mutation. As in previous reports [6, 13, 21], mutations in *KRAS* and *BRAF* in those cases were mutually exclusive, therefore seven (47%) of 15 low-grade serous carcinomas harboured either *KRAS* or *BRAF* mutations. Table 2 summarizes the mutational profiles and clinical features in all the cases. The histopathological appearance and clinical outcome appeared indistinguishable between low-grade tumours with mutations in *KRAS* and *BRAF* and those without, although larger studies are required to confirm this observation. In contrast to a recent study [22] reporting rare *BRAF* mutations in advanced-stage LGCSs, we observed a significantly higher frequency of *BRAF* mutations which occurred in three of 15 (20%, 95% CI 0–40%) advanced-stage LGSCs, and one of

these patients died of the disease 42 months after diagnosis (Table 1, Figure 1). In SBTs (APSTs), a *KRAS* mutation was found in one case and *BRAF* mutations were detected in seven cases. We did not identify mutations in *PIK3CA*, *ARID1A*, *TSPAN11*, *STYK1*, *RNF214*, *DDC* or *SMARCA4* in SBTs (APSTs).

Discussion

Our exome sequencing confirms previous findings that ovarian LGSCs frequently harbour somatic activating mutations in BRAF and KRAS [13–16]. Although rare mutations can be detected in other coding sequences, our data indicate that mutations of BRAF and KRAS represent the most common molecular genetic changes in these tumours and that constitutive activation in the KRAS-BRAF-MEK-MAPK pathway resulting from these mutations plays a pivotal role in their pathogenesis. In fact, active MAPK is more frequently observed in low-grade serous tumours than in high-grade ovarian serous carcinomas, which rarely have mutations in either BRAF or KRAS [23]. This observation suggests that inhibitors of BRAF and MEK could potentially be used to treat patients with advanced-stage LGSCs. Of note, it has been reported that *BRAF* and *KRAS* mutation status is a useful predictor of sensitivity to MEK inhibition in ovarian cancers [21, 24]. This is important because, unlike high-grade serous carcinoma, LGSCs are notorious for their poor response to conventional platinumbased chemotherapeutic regimens. An ongoing phase II trial (GOG 0239) of AZD6244, a MEK inhibitor, should determine whether there is clinical benefit in targeting MEK pathway for patients with LGSC and whether the response is associated with mutations of BRAF and KRAS.

The findings from the current study also highlight the distinctive molecular genetic features of LGSC as compared to other histological subtypes of ovarian cancer. It has previously been proposed that ovarian surface epithelial tumours can be broadly classified into type I and type II tumours [2, 8]. Type I tumours include LGSCs, low-grade endometrioid, clear cell and mucinous carcinomas, and type II tumours are composed, for the most part, of high-grade serous carcinomas. Fundamental differences between the molecular genetic features of type I and type II tumours have been identified in several reports. Those studies concluded that the type I tumours are relatively genetically stable and contain somatic mutations of *KRAS, BRAF, PTEN, PIK3CA CTNNB1, ARID1A* and *PPP2R1A* but rarely *TP53*. In contrast, type II tumours are chromosomally unstable and harbour *TP53* mutations in > 95% of cases; they rarely have the mutations found in the type I tumours [17, 18, 20, 25]. Thus, the exome sequencing of LGSC as reported in this study, together with our previous exome sequencing study in ovarian clear cell carcinoma, provides cogent evidence that the type I tumours are highly heterogeneous and are characterized by a distinct repertoire of mutated genes.

Occasionally, LGSCs appear to contain histopathological elements characteristic of other type I tumours, such as endometrioid or clear cell carcinomas. These 'mixed' tumours present diagnostic and managerial challenges because their behaviour and molecular features are not well understood. Our discovery set contained two such tumours. Mutational analysis of these two cases provided evidence that these histologically mixed tumours molecularly resemble endometrioid and clear cell carcinoma more than LGSC, because they harboured *PIK3CA* or *ARID1A* mutations, which are found in 30% of endometrioid and 50% of clear cell carcinomas, respectively [18–20, 26, 27] (Table 2). Moreover, OV207, which showed endometrioid features, was characterized by a mutator phenotype associated with a mutation in a mismatch-repair gene. Such mutator phenotypes are commonly found in endometrioid carcinomas. As not all LGSCs have mutations in *KRAS* or *BRAF* it is possible that the tumour-promoting functions of *KRAS*- and *BRAF*-mutated genes are conferred by other mutations or epigenetic activation in the same MEK pathway [28]. To

this end, a previous immunohistochemistry study has shown that active (phosphorylated) MAPK is present not only in all low-grade serous tumours with either *KRAS* or *BRAF* mutation but also in 41% of tumours without such mutations [23].

Perhaps the most interesting aspect of our study was the rarity of point mutations. On average, morphologically pure LGSCs averaged 7.5 somatic non-synonymous and splice site mutations/tumour. This number is far lower than in most common tumours of adults, in which 25–75 somatic mutations/tumour are commonly observed. It is lower than in the three other types of gynaecological cancers studied (34 mutations/tumour in ovarian clear cell carcinoma [18], 48 mutations/tumour in high-grade serous carcinoma [17], 45 mutations/ tumour in uterine serous carcinoma), and even lower than in paediatric tumours, such as medulloblastomas [29]. Two independent conclusions can be made from this observation. First, the precursor cells for LGSC must have not replicated much prior to the initiation of tumourigenesis. Second, there must have been relatively few bottlenecks once this initiation occurred. If either of these conclusions were invalid, then a much larger number of mutations— mostly passengers—would have been observed [30]. A corollary of this conclusion is that the ratio of driver gene mutations (those which confer a selective growth advantage) to passenger gene mutations in LGSCs should be higher than is usually observed in other adult tumour types. It will be interesting to see whether the genes mutated at low frequency in LGSCs turn out to be drivers after further genetic and functional analyses.

Our data suggest that LGSCs do not require very many mutational 'hits' to achieve malignancy [31]. It is thereby possible that targeted therapeutic agents, such as those active against BRAF [32], might be particularly effective against these tumours, as they are in chronic myelogenous leukaemia (another type of tumour with a small number of mutational hits). An alternative conclusion is that LGSCs require many more mutations to develop into full-blown malignancy than our sequencing analysis suggests. Although we can exclude frequent amplifications and deletions through our copy number analysis [12], other alterations, such as translocations and epigenetic changes, are yet to be explored. Accordingly, future genome-wide analyses including miRNA profiles, promoter methylation patterns and mRNA sequencing are warranted to study the contribution of molecular alterations other than sequence mutations and DNA copy number to the development of ovarian LGSC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by the Dr Miriam and Sheldon G Adelson Medical Research Foundation, the Virginia and DK Ludwig Fund for Cancer Research, AACR Stand Up To Cancer–Dream Team Translational Cancer Research Grant, and NIH/NCI (Grant Nos CA116184, CA103937, CA129080, CA43460, CA160036, CA1165807 and CA121113).

References

- Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin. 2011; 61:69–90. [PubMed: 21296855]
- 2. Cho KR, Shih Ie M. Ovarian cancer. Annu Rev Pathol. 2009; 4:287–313. [PubMed: 18842102]
- 3. Plaxe SC. Epidemiology of low-grade serous ovarian cancer. Am J Obstet Gynecol. 2008; 198:459, e1–e8. discussion, e8-9. [PubMed: 18395040]

- Grimley PM, Matsuno RK, Rosenberg PS, et al. Qualitative age interactions between low-grade and high-grade serous ovarian carcinomas. Cancer Epidemiol Biomarkers Prev. 2009; 18:2256–2261. [PubMed: 19622723]
- 5. Gershenson DM, Sun CC, Lu KH, et al. Clinical behavior of stage II–IV low-grade serous carcinoma of the ovary. Obstet Gynecol. 2006; 108:361–368. [PubMed: 16880307]
- Dehari R, Kurman RJ, Logani S, et al. The development of high-grade serous carcinoma from atypical proliferative (borderline) serous tumors and low-grade micropapillary serous carcinoma: a morphologic and molecular genetic analysis. Am J Surg Pathol. 2007; 31:1007–1012. [PubMed: 17592266]
- Shvartsman HS, Sun CC, Bodurka DC, et al. Comparison of the clinical behavior of newly diagnosed stages II–IV low-grade serous carcinoma of the ovary with that of serous ovarian tumors of low malignant potential that recur as low-grade serous carcinoma. Gynecol Oncol. 2007; 105:625–629. [PubMed: 17320156]
- Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol. 2004; 164:1511–1518. [PubMed: 15111296]
- 9. Shih Ie M, Kurman RJ. Molecular pathogenesis of ovarian borderline tumors: new insights and old challenges. Clin Cancer Res. 2005; 11:7273–7279. [PubMed: 16243797]
- 10. Ho C-L, Kurman RJ, Dehari R, et al. Mutations of *BRAF* and *KRAS* precede the development of ovarian serous borderline tumors. Cancer Res. 2004; 64:6915–6918. [PubMed: 15466181]
- May T, Virtanen C, Sharma M, et al. Low malignant potential tumors with micropapillary features are molecularly similar to low grade serous carcinoma of the ovary. Gynecol Oncol. 2010; 117:9– 17. [PubMed: 20117829]
- Kuo KT, Guan B, Feng Y, et al. analysis of DNA copy number alterations in ovarian serous tumors identifies new molecular genetic changes in low-grade and high-grade carcinomas. Cancer Res. 2009; 69:4036–4042. [PubMed: 19383911]
- Singer G, Oldt R 3rd, Cohen Y, et al. Mutations in *BRAF* and *KRAS* characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst. 2003; 95:484–486. [PubMed: 12644542]
- Nakayama K, Nakayama N, Kurman RJ, et al. Sequence mutations and amplification of *PIK3CA* and *AKT2* genes in purified ovarian serous neoplasms. Cancer Biol Ther. 2006; 5:779–785. [PubMed: 16721043]
- Sieben NL, Macropoulos P, Roemen GM, et al. In ovarian neoplasms *BRAF*, but not *KRAS*, mutations are restricted to low-grade serous tumours. J Pathol. 2004; 202:336–340. [PubMed: 14991899]
- Mayr D, Hirschmann A, Lohrs U, et al. *KRAS* and *BRAF* mutations in ovarian tumors: a comprehensive study of invasive carcinomas, borderline tumors and extraovarian implants. Gynecol Oncol. 2006; 103:883–887. [PubMed: 16806438]
- TCGA. Integrated genomic analyses of ovarian carcinoma. Nature. 2011; 474:609–615. [PubMed: 21720365]
- Jones S, Wang TL, Shih IM, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science. 2010; 330:228–231. [PubMed: 20826764]
- 19. Wiegand KC, Shah SP, Al-Agha OM, et al. *ARID1A* mutations in endometriosis-associated ovarian carcinomas. N Engl J Med. 2010; 363:1532–1543. [PubMed: 20942669]
- Kuo KT, Mao TL, Jones S, et al. Frequent activating mutations of *PIK3CA* in ovarian clear cell carcinoma. Am J Pathol. 2009; 174:1597–1601. [PubMed: 19349352]
- Pohl G, Ho CL, Kurman RJ, et al. Inactivation of the mitogen-activated protein kinase pathway as a potential target-based therapy in ovarian serous tumors with *KRAS* or *BRAF* mutations. Cancer Res. 2005; 65:1994–2000. [PubMed: 15753399]
- 22. Wong KK, Tsang YT, Deavers MT, et al. BRAF mutation is rare in advanced-stage low-grade ovarian serous carcinomas. Am J Pathol. 2010; 177:1611–1617. [PubMed: 20802181]
- 23. Hsu C-Y, Bristow R, Cha M, et al. Characterization of active mitogen-activated protein kinase in ovarian serous carcinomas. Clin Cancer Res. 2004; 10:6432–6436. [PubMed: 15475429]

- Nakayama N, Nakayama K, Yeasmin S, et al. *KRAS* or *BRAF* mutation status is a useful predictor of sensitivity to MEK inhibition in ovarian cancer. Br J Cancer. 2008; 99:2020–2028. [PubMed: 19018267]
- 25. Ahmed AA, Etemadmoghadam D, Temple J, et al. Driver mutations in *TP53* are ubiquitous in high grade serous carcinoma of the ovary. J Pathol. 2010; 221:49–56. [PubMed: 20229506]
- 26. Campbell IG, Russell SE, Choong DY, et al. Mutation of the *PIK3CA* gene in ovarian and breast cancer. Cancer Res. 2004; 64:7678–7681. [PubMed: 15520168]
- 27. Guan B, Mao TL, Panuganti PK, et al. Mutation and loss of expression of *ARID1A* in uterine lowgrade endometrioid carcinoma. Am J Surg Pathol. 2011; 35:625–632. [PubMed: 21412130]
- Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med. 2004; 10:789– 799. [PubMed: 15286780]
- Parsons DW, Li M, Zhang X, et al. The genetic landscape of the childhood cancer medulloblastoma. Science. 2011; 331:435–439. [PubMed: 21163964]
- Jones S, Chen WD, Parmigiani G, et al. Comparative lesion sequencing provides insights into tumor evolution. Proc Natl Acad Sci USA. 2008; 105:4283–4288. [PubMed: 18337506]
- 31. Knudson AG. Cancer genetics. Am J Med Genet. 2002; 111:96–102. [PubMed: 12124744]
- Yang H, Higgins B, Kolinsky K, et al. RG7204 [PLX4032], a selective BRAFV600E inhibitor, displays potent antitumor activity in preclinical melanoma models. Cancer Res. 2010; 70:5518– 55127. [PubMed: 20551065]
- Burks RT, Sherman ME, Kurman RJ. Micropapillary serous carcinoma of the ovary. A distinctive low-grade carcinoma related to serous borderline tumors. Am J Surg Pathol. 1996; 20:1319–1330. [PubMed: 8898836]

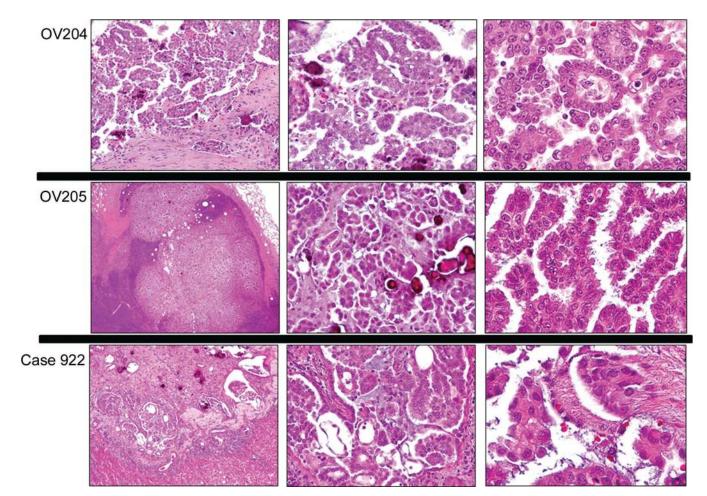


Figure 1.

The morphological features from haematoxylin and eosin-stained sections of three advanced-stage (FIGO stages IIIc and IV), low-grade serous carcinomas of the ovary. All the three cases harboured V600E *BRAF* mutations. (Left panels) Low-magnification views of low-grade serous carcinoma cells metastasizing to the peritoneal wall (OV204 and case 922) or a para-aortic lymph node (OV205). (Middle panels) Medium-magnification views of the tumour cells, exhibiting the characteristic micropapillary architecture with scattered calcification depositions (so-called 'psammoma bodies'). (Right panels) High-magnification views revealing the cytological features of those tumours that are characterized by 'low-grade' or 'grade 1' nuclei, including relatively small and homogeneous nuclear contours and undetectable mitotic figures.

Amino acidMutation γT $p.10A > V$ Missense γT $p.487A > V$ Missense A NA Splice site A $P.316G > S$ Missense $P.249R > W$ $P.316G > S$ Missense $P.249R > W$ $P.386R > C$ Missense A $P.249R > W$ Missense A $P.176A > D$ Missense A $P.176A > MissenseP.176A > MissenseAP.176A > MissenseP.176A > MissenseAP.176A > MissenseP.168A > VAP.168A > VMissenseAP.178 > TP.178 > MissenseA$								
(CDS749.1 $(cubt.) (cubt.) ($		Gene	Transcript Accession No.	Nucleotide (genomic)	Nucleotide (cDNA)	Amino acid (protein)	Mutation type	Confirmed by Sanger
B CCDS32401.1 g.mt/6: 2039965C > T ci.460 > C > T p.487 > V Misense 22 CCDS4308.1 g.mt/6: 2039965C > T IVS8-1G > M Splice site 23 CCDS4308.1 g.mt/1: 8759585G > A IVS8-1G > M Splice site 24 CCDS302.1 g.mt/1: 5253870G > A IVS4-3G > Misense Misense 4 CCDS401.1 g.mt/1: 5253870G > A C4243-4640pCACC Misense Misense 4 CCDS504.1 g.mt/1: 5253870G > A C4243-4640pCACC Misense Misense 6 CCDS504.1 g.mt/1: 2057346T C4243-4640pCACC Misense Misense 7 CCDS109.1 g.mt/1: 1027706C T C4243-4640pCACC Misense Misense 7 CCDS109.1 g.mt/1: 1027706T C C23404D M Misense Misense 7 CCDS109.1 g.mt/1: 1027706C T Misense Misense Misense 7 CCDS108.1 g.mt/1: 1257016C T M Misense Misense Misense 7 CCDS1408.1 g.mt/1: 1257016C T M		ABCD3	CCDS749.1	g.chr1:94656651C>T	c.29C > T	p.10A > V	Missense	Yes
22CCDS43608.1gehr/: 873585.05 × MIVS8-1G > MNASplice site i' CCDS302.1gehr/: 654631440 > ANASplice siteNA i' CCDS302.1gehr/: 654631440 > ANASplice siteNasense i' CCDS302.1gehr/: 5233870G > A $:049 + 5G > A$ NASplice site i' CCDS304.1gehr/: 229348. $:043 + 460 + CAGNARameshifi'CCDS304.1gehr/: 229348.:043 + 460 + CAGNARameshifi'CCDS306.1gehr/: 203748.:043 + 640 + CAGNARameshifi'CCDS306.1gehr/: 12070406 > A:0202 \cdot C + Tp06 R > CNasensei'CCDS306.1gehr/: 12270164 > T:075 \cdot Fp107 > PMasensei'CCDS106.1gehr/: 12270164 > T:030 \cdot F > Tp107 > PMasensei'CCDS306.1gehr/: 12270164 > T:030 \cdot F > Tp107 > PMasensei'CCDS306.1gehr/: 12270164 > T:030 \cdot F > Tp107 > PMasensei'CCDS306.1gehr/: 12270164 > T:030 \cdot F > Tp107 > PMasensei'CCDS306.1gehr/: 12270164 > T:075 \cdot F > Pp107 > Pp107 > Pi'CCDS306.1gehr/: 12270164 > T:075 \cdot F > Pp107 > Pp107 > Pi'CCDS306.1gehr/: 12270164 > T:075 \cdot F > Pp107 > Pp107 > Pi'CCDS306.1gehr/: 12370400 > T:075 $	I I	ACSM2B	CCDS32401.1	g.chr16:20399695C>T	c.1460 > C > T	p.487A > V	Missense	No
'CCDS302.1gend:: 1245400-12dupATCGc.124144dbGCATNAFrameshif $'$ CCDS629.1gend:: 654631440 > AIYS4+3G > ANASpliee siteNisense $'$ CCDS119gent1: 52338706 > Ac.946 > G > A 2160 Spliee siteNisense $'$ CCDS306.1gent1: 2293348c.943 + 46dupCAGCNAFrameshif $'$ CCDS306.1gent1: 2097348c.2902 > C > T 296 % % % %Nisense $'$ CCDS306.1gent1: 12215737 deTc.2022 < Misense	I	ADAM22	CCDS43608.1	g.chr7 : 87595852G > A	IVS8-1G > A	NA	Splice site	Yes
$A\pi3LI$ $CCD8.629.1$ $gent1: 65463144G > A$ $IVS44.3G > A$ $INS4.3G$ $Splite siteAKAPICCD811594.1gent1: 2537370G > Ae.946 > G > Ap.316G > SMisenseARDIACCD811594.1gent1: 26973348e.946 > G > Ap.316G > SMisenseARDIACCD8236.1gent1: 20973348e.945 > G > Ap.316G > SMisenseARDIACCD8306.1gent1: 2097348C > Ce.292.46d ANAPameshiftARTE'CCD8306.1gent1: 12073949C > Te.292.46d ANisenseNisenseARTB'CCD8306.1gent1: 152570164C > Te.22246d ANisenseNisenseA77BB'CCD8306.1gent1: 152570164C > Te.22246ANisenseNisenseA77BB'CCD8306.1gent1: 152570164C > Te.232C > Tp.060V > EMisenseA77BB'CCD8306.1gent1: 125570164C > Te.232C > Tp.060V > EMisenseBRS7'CCD8307.41gent1: 125570164C > Te.232C > Tp.075 > EMisenseBRA7'CCD8303.1gent1: 12570164C > Te.232C > Tp.075 > EMisenseBRS7'CCD8302.1gent1: 122739940 > Te.232C > Tp.075 > EMisenseBRA7'CCD8302.1gent1: 12273940 > Te.232C > Tp.060V > EMisenseBRA7'CCD8302.1gent1: 122733940 > Te.2326 > Tp.060V > EMisense$		ADCY5	CCDS3022.1	g.chr3 : 124554009-12dupATGC	c.1241-44dupGCAT	NA	Frameshift	Yes
$AKAPI$ $CCDS115941$ $geht1: 5233770G \times A$ $p.316G \times S$ $Pisense $ $ARDDIA$ $CCDS2851$ $geht1: 2697348$ - $c.4243-46dupCAGC$ Na Frameshift $ARDDIA$ $CCDS20661$ $geht6: 101207408G \times A$ $c.4243-46dupCAGC$ $Misense$ $Misense$ $ASTE7$ $CCDS20681$ $geht6: 101207408G \times A$ $c.2902 \times C \times T$ $p.968R \times C$ $Misense$ $ASTE7$ $CCDS30681$ $geht6: 101207408G \times T$ $c.2302 \times C \times T$ $p.06R \times C$ $Misense$ $ATPBA$ $CCDS10661$ $geht1: 12570164C \times T$ $c.2302 \times C \times T$ $p.06R \times C$ $Misense$ $ATPBA$ $CCDS108611$ $geht1: 12570164C \times T$ $c.2302 \times C \times T$ $p.1075 \times R$ $Misense$ $BA1AP2L2$ $CCDS108611$ $geht1: 12570164C \times T$ $c.320 \times T$ $p.1075 \times R$ $Misense$ $BA1AP2L2$ $CCDS108611$ $geht1: 122703940 \times T$ $c.11997 \times A$ $p.1075 \times R$ $Misense$ $BA1AP2L2$ $CCDS3863.1$ $geht1: 122703664 \times T$ $c.11997 \times A$ $p.1075 \times R$ $Misense$ $BA1AP2L2$ $CCDS3863.1$ $geht1: 122703664 \times T$ $c.11997 \times A$ $p.1075 \times R$ $Misense$ $BA1AP2L2$ $CCDS3863.1$ $geht1: 122703664 \times T$ $c.2300 \times T$ $p.1075 \times R$ $Misense$ $BA1AP2L2$ $CCDS3863.1$ $geht1: 122703964 \times T$ $c.2300 \times T$ $p.1075 \times R$ $Misense$ $BA1AP2L2$ $CCDS3863.1$ $geht1: 122703960 \times T$ $c.2300 \times T$ $p.1075 \times R$ $Misense$ $C1047207$ $CCDS3864.1$ $geht1: 47630100 \times R$ $c.2300 \times T$ $p.1060 $	1	AK3LI	CCDS629.1	g.chr1 : 65463144G > A	IVS4+3G > A	NA	Splice site	Yes
$ARDIA$ $CCDS268.1$ $geht: 26073548 c.424346upCAGC$ $MarehifiFramehifiASCC3CCDS3046.1geht: 10127408C3 \land Cc.2902 \cdot C \cdot Tp968R \cdot CMisenseASTE/LCCDS306.1geht: 10127408C4 \land Cc.2902 \cdot C \cdot Tp968R \cdot CMisenseATP1B4CCDS1459.1geht: 1123770164C \cdot Tc.23224delANA MisenseATP1B4CCDS1459.1geht: 1123770164C \cdot Tc.2302 \cdot Tp1075 \cdot FMisenseATP2B2CCDS146.1geht: 122730940 \cdot Tc.3202 \cdot Tp1075 \cdot FMisenseBA37CCDS3724.1geht: 122730467 \cdot Tc.3202 \cdot Tp1075 \cdot FMisenseBA37CCDS372.1geht: 122730467 \cdot Tc.3206 \cdot Tp1075 \cdot FMisenseBA37CCDS363.1geht: 122730467 \cdot Tc.1797 \cdot FP107 \cdot FMisenseBA37CCDS363.1geht: 14763010C5 \cdot Tc.1797 \cdot FP102 \cdot FMisenseC160707CCDS363.1geht: 14763010C5 \cdot Tc.1797 \cdot FP102 \cdot FMisenseC160707CCDS363.1geht: 14763010C5 \cdot Tc.3365 \cdot TP102 \cdot FMisenseC160707CCDS363.1geht: 1273343047 \cdot Tc.2306 \cdot TP102 \cdot FMisenseC160707CCDS363.1geht: 12733360350467 \cdot Tc.1947 \cdot FP102 \cdot FMisenseC160707CCDS363.1geht: 12455330467 \cdot TC1924 \cdot FP102 \cdot FP102 \cdot F<$		AKAPI	CCDS11594.1	g.chr17:52538770G > A	c.946 > G > A	p.316G > S	Missense	No
ASCC3CCDS5046.1gente: 101201408C > Ac.2902 > C > Tp.96R > CMissenseASTE/CCDS308.1gent3: 1321577 delTc.2224delANAFramshiftATP1B4CCDS1459.8.1gent3: 13221577 delTc.2224delANAFramshiftATP84CCDS1459.8.1gent1: 152570164C > Tc.320C > Tp.1075 > FMissenseBA17P22CCDS1408.1gent1: 152570164C > Tc.320C > Tp.1075 > FMissenseBA27CCDS108.1gent1: 125770164C > Tc.320C > Tp.1075 > FMissenseBB57CCDS3724.1gent1: 122973994C > Tc.320C > Tp.1076 > DMissenseBB57CCDS3724.1gent1: 1297394G > Tc.1799 > AP.106PC190723CCDS3724.1gent1: 1240306G > Ac.1799 > AMissensePC190724CCDS142gent1: 7455397 Cc.1386 > TMissensePC190725CCDS142gent1: 7455397 Cc.224A GMissensePC190726CCDS143gent1: 7455397 Cc.2024A GPMissenseC190729CCDC142gent1: 7455397 Cc.2024A GPMissenseC190729CCDC142CCDS1951gent1: 7455397 Cc.2024A GPMissenseC190729CCDC142CCDS1951gent1: 7455397 Cc.2024A GPPC190729CCDC142CCDS1951gent1: 7455397 Cc.2024A GPPC190729CCDS1951gent1: 74553897 Cc.2024A GPPP <td></td> <td>ARIDIA</td> <td>CCDS285.1</td> <td>g.chr1 : 26973548- 51dupCAGC</td> <td>c.4243-46dupCAGC</td> <td>NA</td> <td>Frameshift</td> <td>Yes</td>		ARIDIA	CCDS285.1	g.chr1 : 26973548- 51dupCAGC	c.4243-46dupCAGC	NA	Frameshift	Yes
ASTEICCDS 3068.1gents: 13221573 deltc.2224delANAFrameshiftATP1B4CCDS 14508.1gentx: 11939349C > T $c.745C > T$ $p.249R > WMissenseATP1B4CCDS 1066.1gent: 155570164C > Tc.320C > Tp.107S > FMissenseBAJAP2L2CCDS 1066.1gent: 155570164C > Tc.320C > Tp.107S > FMissenseBAS7CCDS 3724.1gent: 122973994C > Tc.320C > Tp.106N > IMissenseBAS7CCDS 3724.1gent: 12979394C > Tc.1397 > Ap.166N > IMissenseBAS7CCDS 368.2gent: 12979394C > Tc.1397 > Ap.166N > IMissenseBAS7CCDS 368.2gent: 1297394C > Tc.1397 > Ap.166N > IMissenseC130723CCDS 368.2gent: 1297394G > Tc.1397 > Ap.166N > IMissenseC1407106CCDS 368.1gent: 1476310G > Ac.335G > Tp.360 > IMissenseC1407107CCDS 368.1gent: 1453341G > Tc.335G > Tp.126 > WMissenseC1407106CCDS 368.1gent: 1453391G > Tc.335G > Tp.366A > TMissenseC1407106CCDS 368.1gent: 1445301G > Tc.335G > Tp.366A > Tp.366N > Ip.366N > IC1407106CCDS 368.1gent: 10338997 < C$		ASCC3	CCDS5046.1	g.chr6:101207408G > A	c.2902 > C > T	p.968R > C	Missense	No
ATP1B4CCDS14598.1 $chTr1B4$ CCDS14598.1 $chTr1113270164C > T$ $c732C > T$ $p249F > W$ Missense $ATPB2$ CCDS1066.1 $cht1:15270164C > T$ $c:320C > T$ $p.107S > F$ Missense $BAAP2L2$ CCDS106.1 $cht1:15270164C > T$ $c:320C > T$ $p.107S > F$ Missense $BBS7$ CCDS3724.1 $cht2:368230706 > T$ $c:327 > C > A$ $p.176A > D$ Missense $BBS7$ CCDS3724.1 $cht2:12973994C > T$ $c:1797 > A$ $p.106M > I$ Missense $BBS7$ CCDS363.1 $cht1:1297396A > T$ $c:1797 > A$ $p.106M > I$ $MissenseDC1007D2CCDS368.2cht1:129306A > Tc.1797 > AMissenseP.1000 > IC1007D71CCDS176.1cht1:1475010G > AVS1-3deTNA > P.129G > WMissenseC1007D71CCDS142CCDS142cht1:174553397 > C.2024 > Tp.129G > WMissenseC1007D74CCDS142CCDS142CCDS142p.129G > WMissenseC1007M71CCDS142CCDS142p.129G > Wp.129G > WMissenseC1007M71CCDS142CCDS142p.12457 > Tp.1287 > MissenseCCDC142CCDS142p.1$		ASTEI	CCDS3068.1	g.chr3 : 132215737	c.2224delA	NA	Frameshift	No
ATP8B2CCDS1066.1g.chr1:152570164C>Tc320C>Tp.1078>FMisenseBA1AP2L2CCDS43018.1g.chr1:152570164>Tc527>C>Ap.176A>DMisenseBA1AP2L2CCDS3018.1g.chr1:122973994C>Tc518G>Ap.506M>1MisenseBB57CCDS3724.1g.chr1:122973994C>Tc.1518G>Ap.506M>1MisenseBR4FCCDS368.2g.chr1:122973994C>Tc.1799T>Ap.506M>1MisenseBR4FCCDS368.1g.chr1:12093065A>Tc.1799T>Ap.506M>1MisenseC130723CCDS968.2g.chr1:14009065A>Tc.1794CAp.84Q>XMisenseC130710CCDS116.1g.chr1:7455304G>Tc.2530C>Tp.129G>WMisenseC1407106CCDS1945.1g.chr1:7455304G>Tc.2024A>Gp.129G>WMisenseC107071CCDS195g.chr1:7455304G>Tc.2024A>Gp.129G>WMisenseC107071CCDS196g.chr1:7455304G>Tc.2024A>Gp.129G>WMisenseC70772CCDS1951g.chr1:7455304G>Tc.2024A>Gp.129G>WMisenseC70773CCDS196g.chr1:74353897Cc.2024A>Gp.129G>WMisenseC70774CCDC33CCDS1961g.chr1:147AAc.194>C>Tp.129G>WMisenseC70774CCDC34CCDS1961g.chr1:10037897C>Tc.1157C>Ap.168A>VMisenseC70773CCDS1961g.chr1:10037897C>Tc.104+A>Tp.565N>1MisenseC70774CCDS191g.chr1:10037897C>Tc.104+A>Tp.565N>1		ATP1B4	CCDS14598.1	g.chrX:119393449C > T	c.745C > T	p.249R > W	Missense	Yes
BAIAP2L2 CCDS43018.1 Gent-2: 36823070G > T c. 537 > C > A p.176 > D Missense BBS7 CCDS3724.1 g.chr4: 122973994C > T c.1518G > A p.506M > I Missense BBS7 CCDS3724.1 g.chr4: 122973994C > T c.1518G > A p.506M > I Missense BRAF CCDS363.1 g.chr1: 120979605A > T c.1799T > A p.506M > I Missense BRAF CCDS368.2 g.chr13: 38506336 delA INS1-3deT NA Splice site C140r106 CCDS9368.2 g.chr13: 1355034G > T c.2530C > T p.844Q > X Nonsense C140r116 g.chr13: 74553807 > C c.23867 T p.844Q > X Nonsense C140r1106 CCDS142 g.chr13: 74553807 > C c.23867 T p.164 > X Nonsense C140r116 g.chr13: 74553807 > C c.23867 T p.168 > X Missense D C2DC742 CCDS142 g.chr13: 74553807 > C c.2024 > G p.675 > Nissense D C2DC733 CCDS142 g.chr13: 7457 > T c.214 > C D D		A TP8B2	CCDS1066.1	g.chr1 : 152570164C > T	c.320C > T	p.107S > F	Missense	Yes
BBS7CCDS3724.1g.chrf.: 122973994 c. $1518G$ $p.506M$ MisenseBRAFCCDS363.1g.chrf.: 14009605 r. 17997 $p.600V$ MisenseBRAFCCDS368.2g.chrf.: 14009605 r. 17997 $p.600V$ MisenseC13 $ort23$ CCDS368.1g.chr1: 14009605 $p.c1793$ $p.600V$ MisenseC13 $ort23$ CCDS368.1g.chr1: $14763010G$ $p.2365$ $p.84Q$ $Nonsense$ C1 $ortVPI$ CCDS161.1g.chr1: $7455304G$ $p.2365$ $p.29G$ $Misense$ C1 $OTVFI$ CCDS1945.1g.chr1: $7455304G$ $p.2024$ $p.129G$ $Misense$ C1 $OTVFI$ CCDS1945.1g.chr1: $7455304G$ $p.2024$ $p.675N$ $Misense$ C1 $OTVFI$ CCDS1945.1g.chr1: $7455304G$ $p.2024$ $p.675N$ $Misense$ C1 $OTVFI$ CCDS1945.1 $g.chr1: 7455304G$ $p.2024$ $p.675N$ $Misense$ C1 $OTVFI$ CCDS1945.1 $g.chr1: 7455304G$ $p.2024$ $p.675N$ $Misense$ C1 $OTVFI$ CCDS1945.1 $g.chr1: 7455304G$ $p.2024$ $p.675N$ $Misense$ C1 $OTV2$ CCDS194.1 $g.chr1: 7455304G$ $p.2024$ $p.705S$ $Misense$ CDC23CCDS1450.1 $g.chr1: 700378997C$ $p.2024$ $p.705S$ $Misense$ CDDC142CCDS111 $g.chr1: 700378997C$ $p.2025$ $p.705S$ $Misense$ CDC23CCDS1450.1 $g.chr1: 700378997C$ $p.2024$ $p.705S$ $Misense$ CDC27CCDS1690.1 $g.chr1: 70037897C$ <td></td> <td>BAIAP2L2</td> <td>CCDS43018.1</td> <td>g.chr22 : 36823070G > T</td> <td>c.527 > C > A</td> <td>p.176A > D</td> <td>Missense</td> <td>No</td>		BAIAP2L2	CCDS43018.1	g.chr22 : 36823070G > T	c.527 > C > A	p.176A > D	Missense	No
BRAF CCDS5863.1 g.chr7:14009605A>T c.1797>A p.600V>E Misense C13or123 CCDS968.2 g.chr13:38506336 delA IVS1-3delT NA Splice site C13or123 CCDS968.1 g.chr13:38506336 delA IVS1-3delT NA Splice site C13or124 CCDS968.1 g.chr13:1476310G>A c.2530C>T p.844Q>X Nonsense C14or106 CCDS1945.1 g.chr17:14553304G>T c.2530C>T p.844Q>X Nonsense C2DC142 CCDS1945.1 g.chr17:14553304G>T c.385G>T p.129G>W Missense C2DC142 CCDS1945.1 g.chr17:14553304G>T c.385G>T p.120G>W Missense C2DC142 CCDS143 g.chr13:1457C>T c.2024AG p.65N>T Missense CCDC142 CCDS140 g.chr13:1037897C>T c.2014AG p.65N>T Missense CCDC133 CCDS140 g.chr13:1037897C>T g.chr14:1077 p.168A>V Missense CCDC133 CCDS141 g.chr13:1427C>T g.149C>T p.168A>V Missense		BBS7	CCDS3724.1	g.chr4 : 122973994C > T	c.1518G > A	p.506M > I	Missense	Yes
C13ort23 CCDS9368.2 g.chr13: 38506336 delA IVS1-3deT Na Splice site C14ort106 CCDS9684.1 g.chr14: 44763010G > A c.2530C > T p.844Q > X Nonsense C14ort106 CCDS9684.1 g.chr14: 74555304G > T c.2530C > T p.129G > W Nissense C14ort105 CCDS145.1 g.chr17: 74555304G > T c.385G > T p.129G > W Missense CCDC142 CCDS145.1 g.chr17: 745553897 > C c.2024 > G p.129G > W Missense CCDC33 CCDS104.1 g.chr15: 72414457 > T c.204 > G p.168A > V Missense CCDC33 CCDS165.1 g.chr11: 100378997 > T c.2014 > C p.168A > V Missense CCDC73 CCDS1509.1 g.chr11: 100378997 > T c.303 > T p.168A > V Missense CCDC74 CCDS1509.1 g.chr11: 100378997 > T c.303 > T p.168A > V Missense CCDC74 CCDS150 g.chr11: 100378997 > T c.1694 > A > T p.168A > V Missense CDC27 CCDS1891.1 g.chr11: 10037897 > T c.		BRAF	CCDS5863.1	g.chr7 : 140099605A > T	c.1799T > A	p.600V > E	Missense	Yes
C14ont106 $CCDS9684.1$ $g.chr14: 4763010G > A$ $c.2530C > T$ $p.844Q > X$ Nonsense $C1QTNF1$ $CCDS11761.1$ $g.chr17: 7455304G > T$ $c.385G > T$ $p.129G > W$ Missense $C1QTNF2$ $CCDS1945.1$ $g.chr17: 74553304G > T$ $c.385G > T$ $p.129G > W$ Missense $CCDC142$ $CCDS1945.1$ $g.chr17: 74553304 > C$ $c.2024A > G$ $p.705 > V$ Missense $CCDC742$ $CCDS145.1$ $g.chr15: 72414457C > T$ $c.2024A > G$ $p.7055 > L$ Missense $CCDC73$ $CCDS765.1$ $g.chr15: 72414457C > T$ $c.2114 > C > T$ $p.7055 > L$ Missense $CCDC76$ $CCDS765.1$ $g.chr15: 124147A$ $c.2114 > C > T$ $p.7055 > L$ Missense $CCDC76$ $CCDS765.1$ $g.chr15: 124147C > T$ $c.1064 > A > T$ $p.168A > V$ Missense $CDC76$ $CCDS765.1$ $g.chr15: 12457C > T$ $p.168A > V$ Missense $p.7055 > L$ $CDC76$ $CCDS765.1$ $g.chr15: 42571114T > A$ $c.1694 > A > T$ $p.168A > V$ Missense $CDC77$ $CCDS765.1$ $g.chr17: 42571114T > A$ $c.1694 > A > T$ $p.168A > V$ Missense $CDC77$ $CCDS10$ $g.chr17: 42571114T > A$ $c.1694 > A > T$ $p.168A > V$ Missense $CDC77$ $CCDS100.1$ $g.chr17: 42571114T > A$ $c.1694 > A > T$ $p.168A > V$ Missense $CD192$ $CCD21$ $CCDS100.1$ $g.chr17: 4257114T > A$ $p.168A > V$ $p.17P > T$ $p.17P > T$ $CD192$ $CCD191$ $g.chr17: 4078765C > CCDS364$		C13orf23	CCDS9368.2	g.chr13:38506336 delA	IVS1-3deIT	NA	Splice site	No
CIQTVFI CCDS11761.1 g.chr17:7455304G>T c.385G>T p.129G>W Missense CCDC142 CCDS1945.1 g.chr17:74553389T>C c.385G>T p.675N>S Missense CCDC73 CCDS1945.1 g.chr15:741457C>T c.2024A>G p.675N>S Missense CCDC73 CCDS13 g.chr15:72414457C>T c.2014>C p.675N>S Missense CCDC73 CCDS1180.1 g.chr15:72414457C>T c.2014>C p.675N>L Missense CCDC74 CCDS150.1 g.chr15:72414457C>T c.2014>C p.66SN>L Missense CCDC75 CCDS150.1 g.chr15:0378997C>T c.2014>C p.66A>N Missense CCDC76 CCDS150.1 g.chr15:10378997C>T c.303C>T Missense p.66A>N CCDC77 CCDS1891.1 g.chr15:037897C>T c.303C>T p.66A>N Missense CCD194 CCDS3505.1 g.chr18:23827463G>T c.1694>A>T p.617P>T Missense CD194 CCDS35085.1 g.chr6:70913458C>A c.1694>A>T p.616P>T Missense <tr< td=""><td></td><td>C14orf106</td><td>CCDS9684.1</td><td>g.chr14:44763010G > A</td><td>c.2530C > T</td><td>p.844Q > X</td><td>Nonsense</td><td>Yes</td></tr<>		C14orf106	CCDS9684.1	g.chr14:44763010G > A	c.2530C > T	p.844Q > X	Nonsense	Yes
<i>CCDC142</i> CCDS1945.1 g.chr15: 74553397 > C C.2024A > G p.675N > S Missense <i>CCDC33</i> CCDS14 g.chr15: 72414457C > T c.2114 > C > T p.7055 > L Missense <i>CCDC33</i> CCDS765.1 g.chr15: 72414457C > T c.2114 > C > T p.7055 > L Missense <i>CCDC76</i> CCDS765.1 g.chr15: 124147 > A c.2114 > C > T p.168A > V Missense <i>CDC77</i> CCDS11509.1 g.chr17: 42571114T > A c.1694 > A > T p.168A > V Missense <i>CDC27</i> CCDS11509.1 g.chr17: 42571114T > A c.1694 > A > T p.655N > I Missense <i>CDL27</i> CCDS11891.1 g.chr17: 42571114T > A c.1694 > A > T p.656N > I Missense <i>CDL27</i> CCDS11891.1 g.chr17: 42571114T > A c.1694 > A > T p.365N > I Missense <i>CDL94</i> CCDS1891.1 g.chr17: 4257114T > A c.1694 > A > T p.365N > I Missense <i>CDL94</i> CCDS1891.1 g.chr18: 238274636 > T c.1849C > Missense p.617P > T Missense <i>CDL94</i> CCDS3651.1		CIQTNFI	CCDS11761.1	g.chr17:74555304G > T	c.385G > T	p.129G > W	Missense	Yes
<i>CCDC33</i> CCDS42058.1 g.chr15: 72414457C>T c.2114>C p.705S>L Missense <i>CCDC76</i> CCDS765.1 g.chr1: 100378997C>T c.503C>T p.168A>V Missense <i>CCDC77</i> CCDS11509.1 g.chr1: 100378997C>T c.503C>T p.168A>V Missense <i>CDC27</i> CCDS11509.1 g.chr17: 42571114T>A c.1694>A>T p.168A>V Missense <i>CDL27</i> CCDS11509.1 g.chr17: 42571114T>A c.1694>A>T p.168A>V Missense <i>CDL94</i> CCDS11509.1 g.chr17: 42571114T>A c.1694>A>T p.386T>K Missense <i>CDL94</i> CCDS3505.1 g.chr18: 23827463G>T c.1157C>A p.386T>K Missense <i>CDL94</i> / CCDS3505.1 g.chr6: 70913458C>A c.1849C>A p.617P>T Missense <i>CDL94</i> / CCDS3505.1 g.chr6: 70913458C>A c.1849C>A p.617P>T Missense <i>CDL94</i> / CCDS3505.1 g.chr6: 70913458C>A c.1849C>A p.616F>T Missense <i>CDL94</i> / CCDS3505.1 g.chr6: 70913458C>A c.721G>C p.241E>Q<		CCDC142	CCDS1945.1	g.chr2 : 74555389T > C	c.2024A > G	p.675N > S	Missense	Yes
<i>CCDC76</i> CCDS765.1 g.chr1:10037897C>T c.503C>T p.168A>V Missense <i>CDC27</i> CCDS11509.1 g.chr17:42571114T>A c.1694>A>T p.565N>1 Missense <i>CDC27</i> CCDS11891.1 g.chr17:42571114T>A c.1694>A>T p.565N>1 Missense <i>CDL24</i> CCDS11891.1 g.chr18:238274636>T c.1157C>A p.386T>K Missense <i>CDL941</i> CCDS1891.1 g.chr6:70913458C>A c.1849C>A p.316T>K Missense <i>CDL941</i> CCDS3085.1 g.chr7:1043458C>A c.1849C>A p.617P>T Missense <i>CDL941</i> CCDS3085.1 g.chr5:104807455C>C c.3849C>A p.617P>T Missense <i>DDC</i> CCD1941 g.chr5:104807455C>C c.721G>C p.241E>Q Missense <i>DDC</i> CCDS33081.1 g.chr7:50564474G>A c.496C>T p.166R>W Missense <i>DDC</i> CCDS3381.1 g.chr13:41678A c.2560G>T p.165R>W Missense		CCDC33	CCDS42058.1	g.chr15:72414457C>T	c.2114 > C > T	p.705S > L	Missense	No
CDC27 CCDS11509.1 g.chr17:42571114T>A c.1694>A>T p.565N>1 Missense CDH2 CCDS11891.1 g.chr18:23827463G>T c.1157C>A p.386T>K Missense CDH2 CCDS1891.1 g.chr18:23827463G>T c.1157C>A p.386T>K Missense COL19A1 CCDS36511 g.chr6:70913458C>A c.1849C>A p.386T>K Missense CVL22 CCDS35085.1 g.chr9:104807455G>C c.731G>C p.617P>T Missense DDC CCDS3511.1 g.chr7:5056474G>A c.731G>C p.241E>Q Missense DDC CCDS3511.1 g.chr7:5056474G>A c.496C>T p.166R>W Missense		CCDC76	CCDS765.1	g.chr1 : 100378997C > T	c.503C > T	p.168A > V	Missense	Yes
CDH2 CCDS11891.1 g.chr18: 238274636 > T c.1157C > A p.386T > K Missense COL19A1 CCDS4970.1 g.chr6: 70913458C > A c.1849C > A p.617P > T Missense COL19A1 CCDS3685.1 g.chr9: 104807455G > C c.1849C > A p.617P > T Missense CYLC2 CCDS35085.1 g.chr9: 104807455G > C c.721G > C p.241E > Q Missense DDC CCDS3511.1 g.chr7: 50564474G > A c.496C > T p.166R > W Missense DBC CCDS3511.1 g.chr7: 50564474G > A c.496C > T p.166R > W Missense DBC CCDS331.1 g.chr1: 50564474G > A c.2560G > T p.854A > S Missense		CDC27	CCDS11509.1	g.chr17:42571114T > A	c.1694 > A > T	p.565N > I	Missense	No
COL19A1 CCDS 4970.1 g.chr6: 70913458C > A c.1849C > A p.617P > T Missense CYLC2 CCDS35085.1 g.chr9: 104807455G > C c.721G > C p.241E > Q Missense DDC CCDS5511.1 g.chr7: 50564474G > A c.496C > T p.166R > W Missense DDC CCDS331.1 g.chr13: 41678241G > T c.2560G > T p.854A > S Missense		CDH2	CCDS11891.1	g.chr18:23827463G > T	c.1157C > A	p.386T > K	Missense	Yes
CYLC2 CCDS35085.1 g.chr9:104807455G > C c.721G > C p.241E > Q Missense DDC CCDS5511.1 g.chr7:5056477G > A c.496C > T p.166R > W Missense DGKH CCDS9381.1 g.chr13:41678241G > T c.2560G > T p.854A > S Missense		COL 19A I	CCDS4970.1	g.chr6 : 70913458C > A	c.1849C > A	p.617P > T	Missense	Yes
DDC CCDS5511.1 g.chr7: 50564474G > A c.496C > T p.166R > W Missense DGKH CCDS9381.1 g.chr13: 41678241G > T c.2560G > T p.854A > S Missense	r .	CYLC2	CCDS35085.1	g.chr9 : 104807455G > C	c.721G > C	p.241E > Q	Missense	Yes
DGKH CCDS9381.1 g.chr13:41678241G>T c.2560G>T p.854A>S Missense		DDC	CCDS5511.1	g.chr7 : 50564474G > A	c.496C > T	p.166R > W	Missense	Yes
	r	DGKH	CCDS9381.1	g.chr13:41678241G>T	c.2560G > T	p.854A > S	Missense	Yes

J Pathol. Author manuscript; available in PMC 2012 November 21.

\$watermark-text

Sample	Gene	Transcript Accession No.	Nucleotide (genomic)	Nucleotide (cDNA)	Amino acid (protein)	Mutation type	Confirmed by Sanger
OV202PT	EMR3	CCDS12315.1	g.chr19:14591285T > C	IVS15-2A > G	NA	Splice site	Yes
OV208PT	SHAXE	CCDS8341.1	g.chr11:107886124 G > C	c.5320C > G	p.1774L > V	Missense	Yes
OV209PT	FAM65C	CCDS13431.2	g.chr20:48646191G > A	c.1795C > T	p.599P > S	Missense	Yes
OV208PT	FBNI	CCDS32232.1	g.chr15:46692561C>T	c.185G > A	p.62R > H	Missense	Yes
OV204PT	FSTL5	CCDS3802.1	g.chr4:162678870G > A	c.1210C > T	p.404R > C	Missense	Yes
OV206PT	GNRH2	CCDS13040.1	g.chr20 : 2974356- 60dupGCCCC	c.337-41dupGCCCC	NA	Frameshift	No
OV209PT	HEPACAM	CCDS8456.1	g.chr11:124299968C > T	c.293G > A	p.98R > H	Missense	Yes
OV205PT	IP6K2	CCDS2777.1	g.chr3 : 48701018G > A	c.973C > T	p.325R > C	Missense	Yes
OV209PT	KCN112	CCDS11219.1	g.chr17 : 21260469G > A	c.1222 > G > A	p.408A > T	Missense	No
OV208PT	KIFC2	CCDS6427.1	g.chr8 : 145663014C > G	c.163C > G	p.55L > V	Missense	Yes
OV203PT	KLHL11	CCDS11411.1	g.chr17:37265006G > T	c.639C > A	p.213H > Q	Missense	Yes
OV209PT	APRP	CCDS30862.1	g.chr1:151000349G > A	c.1661G > A	p.554R > Q	Missense	Yes
OV209PT	KRAS	CCDS8703.1	g.chr12:25289551C>T	c.35G > A	p.12G > D	Missense	Yes
OV209PT	LAMP2	CCDS14600.1	g.chrX:119457081C > T	c.1189G > A	p.397V > I	Missense	Yes
OV205PT	LILRAZ	CCDS12900.1	g.chr19:59779118C > T	c.985C > T	p.329Q > X	Nonsense	Yes
OV209PT	LRIGI	CCDS33783.1	g.chr3 : 66513792G > A	c.2954C > T	p.985A > V	Missense	Yes
OV209PT	TLDAT	CCDS32348.1	g.chr16:179998C > T	c.944 > G > A	p.315R > Q	Missense	No
OV205PT	MARCO	CCDS2124.1	g.chr2:119456294C > T	c.994C > T	p.332R > X	Nonsense	Yes
OV203PT	MCM7	CCDS5683.1	g.chr7 : 99533212C > T	c.1078G > A	p.360G > R	Missense	Yes
OV208PT	LHYM 7	CCDS9601.1	g.chr14 : 22968919G > T	c.1043C > A	p.348S > Y	Missense	Yes
OV202PT	NCKI	CCDS3092.1	g.chr3 : 138149854- 6delAAA	c.1003-5delAAA	NA	In-frame deletion	Yes
OV209PT	NLRP3	CCDS1632.1	g.chr1 : 245653904G > A	c.536G > A	p.197S > N	Missense	Yes
OV209PT	OR6K2	CCDS30902.1	g.chr1 : 156936939A > T	c.128T > A	p.43L > Q	Missense	Yes
OV204PT	PBXI	CCDS1246.1	g.chr1 : 163055986C > T	c.1051C > T	p.351Q > X	Nonsense	Yes
OV209PT	PCDHG	CCDS4260.1	g.chr5 : 140792593G > A	c.2083G > A	p.695V > I	Missense	Yes
OV209PT	PHF20	CCDS13268.1	g.chr20:33968975G > T	c.1981G > T	p.661E > X	Nonsense	Yes
OV208PT	PIK3CA	CCDS43171.1	g.chr3 : 180404242G > A	c.1030G > A	p.344V > M	Missense	Yes
OV205PT	PNMA5	CCDS14718.1	g.chrX:151910416C > A	c.383G > T	p.128S > I	Missense	Yes

Jones et al.

\$watermark-text

\$watermark-text

\$watermark-text

Sample	Gene	Transcript Accession No.	Nucleotide (genomic)	Nucleotide (cDNA)	Amino acid (protein)	Mutation type	Confirmed by Sanger
OV205PT	длоd	CCDS33833.1	g.chr3 : 122691680A > T	c.2788T > A	p.930S > T	Missense	Yes
OV208PT	PRKRA	CCDS2279.1	g.chr2:179005230G > A	IVS7-3 > C > T	NA	Splice site	No
OV209PT	PRRXI	CCDS1290.1	g.chr1 : 168971937C > G	c.724C > G	p.242P > A	Missense	Yes
OV208PT	<i>RBL2</i>	CCDS10748.1	g.chr16:52058534C > T	c.1927C > T	p.643P > S	Missense	Yes
OV208PT	REPS2	CCDS14180.2	g.chrX:16980489G > A	c.971G > A	p.324 W > X	Nonsense	Yes
OV209PT	RNF214	CCDS41720.1	g.chr11:116614795G > T	c.376G > T	p.126E > X	Nonsense	Yes
OV208PT	d 8d001S	CCDS30666.1	g.chr1 : 33064901C > G	c.614C > G	p.205S > C	Missense	Yes
OV208PT	TSAS	CCDS9170.1	g.chr12 : 112350202A > G	c.32A > G	p.11Q > R	Missense	Yes
OV205PT	SLC9A6	CCDS44003.1	g.chrX:134908689T > A	c.689 > T < A	p.230M > K	Missense	No
OV209PT	SPA TA5	CCDS3730.1	g.chr4 : 124068326G > T	c.251G > T	p.84R > L	Missense	Yes
OV208PT	dOdS	CCDS11551.1	g.chr17:45054368C > T	c.139G > A	p.47E > K	Missense	Yes
OV208PT	SRCAP	CCDS10689.2	g.chr16 : 30638999C > T	c.2833C > T	p.945R > X	Nonsense	Yes
OV203PT	SRP72	CCDS3506.1	g.chr4 : 57052353G > A	V < 01-21SVI	NA	Splice site	Yes
OV203PT	STYKI	CCDS8629.1	g.chr12 : 10668616- 18delAAA	c.827-29delTTT	NA	In-frame deletion	Yes
OV205PT	TCF25	CCDS10987.1	g.chr16:88478529 dupA	c.393dupA	NA	Frameshift	No
OV204PT	<i>TMEM202</i>	CCDS32287.1	g.chr15:70486577T > C	c.584T > C	p.195L > P	Missense	Yes
OV208PT	TNNI3K-FPGT	CCDS663.1	g.chr1 : 74442707A > T	c.388A > T	p.130I > F	Missense	Yes
OV204PT	OdL	CCDS1643.1	g.chr2:1523465C > A	c.2711C > A	p.904T > N	Missense	Yes
OV203PT	TRIM31	CCDS34374.1	g.chr6:30179332G > A	c.1238C > T	p.413T > I	Missense	Yes
OV209PT	TSG101	CCDS7842.1	g.chr11:18480703de1A	IVS6-3delT	NA	Splice site	Yes
OV204PT	TSPAN11	CCDS31765.1	g.chr12:31008218C > T	c.275C > T	p.92T > M	Missense	Yes
OV209PT	TSPAN11	CCDS31765.1	g.chr12:31027266G > A	c.616G > A	p.206G > R	Missense	Yes
OV204PT	VDR	CCDS8757.1	g.chr12:46545141G > C	c.233 > C > G	p.78A > G	Missense	No
OV208PT	WWTRI	CCDS3144.1	g.chr3 : 150857512G > T	c.272C > A	p.91P > H	Missense	Yes
OV203PT	XIRP2	CCDS42769.1	g.chr2:167814105G > C	c.7957G > C	p.2653D > H	Missense	Yes
OV208PT	ZFYVE16	CCDS4050.1	g.chr5 : 79769548G > C	c.1288G > C	p.430E > Q	Missense	Yes
OV208PT	ZFYVE16	CCDS4050.1	g.chr:579769674G > A	c.1414G > A	p.472D > N	Missense	Yes
OV209PT	ZNF572	CCDS6354.1	g.chr8 : 126057095A > T	c.32A > T	p.11D > V	Missense	Yes

\$watermark-text

\$watermark-text

Page 12

\$watermark-text

 * All coordinates refer to the human reference genome hg18 release (NCBI 36.1, March 2006). NA, not applicable.

Jones et al.

2
θ
Q
a'

Summary of mutational analysis of the 15 genes selected for validation

									1																	
C14orf106	wt	wt	wt	wt	wt	wt	844Q/X	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	1M	1M	wt						
DDC	wt	wt	hwt	hwt	hwt	39R/W	166R/W	hut	wt	1W	1W	wt														
PIK3C2A	wt	wt	wt	wt	wt	572R/X	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
PPP2R1A	wt	wt	wt	wt	wt	183R/Q	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
RBL2	wt	wt	wt	wt	wt	wt	643P/S	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
SPATA5	wt	wt	wt	wt	wt	wt	wt	84R/L	wt	wt	wt	wt	wt	wt	wt	wt	wt									
SMARCA4	wt	wt	wt	wt	wt	539R/C	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
RNF214	wt	wt	wt	wt	wt	239R/H	wt	126E/X	wt	wt	wt	wt	wt	wt	wt	wt	wt									
CCDC76	wt	wt	wt	168A/V	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
STYKI	wt	827delTTT	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
TSPANII	wt	wt	92T/M	wt	wt	wt	wt	206G/R	wt	wt	wt	wt	wt	wt	wt	wt	wt									
ARIDIA	wt	wt	wt	wt	wt	5543insG; 6415delC	4247ins CAGC	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
PIK3CA	wt	wt	wt	wt	wt	88R/Q	344V/M	wt	wt	wt	wt	345N/K	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt	wt
BRAF	wt	wt	600V/E	600V/E	wt	wt	wt	wt	wt	wt	600V/E	wt	600V/E	600V/E	600V/E	600V/E	600V/E	wt	wt	600V/E						
KRAS	wt	wt	wt	wt	wt	wt	wt	12G/D	wt	wt	wt	wt	wt	wt	12G/V	12G/D	12G/D	wt	wt	wt	wt	wt	wt	12G/D	wt	wt
follow up	LWD 74 m	N/A	LWD 60 m	DOD 42 m	LWD 26 m	N/A	DOD 5 m	LWD 54 m	LWD 60 m	LWD 3 m	LWD 23 m	DOD 35 m	N/A	LWD 12 m	LWD 16 m	LWD 10 m	DOD 14 m	ND 24 m	ND 22 m	N/A	ND 26 m	ND 32 m	ND 20 m	N/A	ND 8m	LWD 90 m
stage	IIIC	IV	IIIC	IIIC	IIIC	IC	IV	IIIC	IIIB	IIIC	Π	IIIC	IIIC	шс	IIIC	IIIC	IIIC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
age	32	44	46	72	35	49	49	59	25	45	33	62	45	33	48	56	46	51	54	35	25	37	47	41	67	39
diagnosis	LGS	SDJ	TGS	TGS	TGS	LG (S+EM)	LG (S+CC)	TGS	LGS	TGS	SBT^{*}	SBT^{*}	SBT													
case	OV202	OV203	OV204	OV205	OV206	OV207	OV208	OV209	543	10089	922	116	1107	406	610	701	609	976	832	725	925	1017	869	345	623	485
set	discovery	discovery	discovery	discovery	discovery	discovery	discovery	discovery	validation	validation	validation	validation	validation	validation	validation	validation	validation									
<u> </u>																										

LGS: low-grade serous carcinoma; SBT: serous borderline tumor; EM: endometrioid; CC: clear cell; S: serous; N/A: not available; DOD: dead of disease; LWD: live with disease; ND: no evidence of disease; wt: wild-type.

 $\overset{*}{}_{\rm containing non-invasive low-grade (micropapillary) serous carcinoma.$