BRAF mutations in low-grade serous ovarian cancer and response to BRAF inhibition

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ABSTRACT

Purpose: Low-grade serous ovarian carcinoma (LGSC) responds poorly to chemotherapy and is characterised by activating mutations in the RAS-MAPK pathway, including oncogenic *BRAF*. However, response to BRAF inhibitors is tumor-type specific. Significant improvement in survival is seen in patients with *BRAF*-mutant melanoma, but other cancer types, such as colorectal cancers, are generally less sensitive. We examined the frequency and characteristics of *BRAF*-mutated LGSC and describe response to treatment with BRAF inhibitors.

Patients and Methods: Mutations were assessed in LGSC (n=65) using targeted, exome and whole genome sequencing. Patient characteristics, treatment and clinical outcome were assessed, with a median follow-up time of more than 5 years. BRAF inhibitors were trialed in two patients with a somatic $BRAF^{V600E}$ mutation, one on Dabrafenib monotherapy, monitored clinically, biochemically (CA125 levels) and with PET imaging. Expression of $BRAF^{V600E}$ protein in this patient was assessed by immunohistochemistry.

Results: Amongst LGSC cases, 9/65 (13.8%) had a somatic *BRAF* mutation. Of the nine cases with *BRAF* mutation-positive LGSC, four relapsed with progressive disease and did not respond to conventional chemotherapy. Two of the patients progressed quickly and died due to progression of their disease, and two received targeted treatment. Two patients with $BRAF^{V600E}$ mutation received BRAF inhibitors at relapse and both achieved durable responses.

Conclusion: *BRAF* mutations are not uncommon in patients with LGSC and should be routinely tested as BRAF inhibitors can be an effective treatment for these patients. The results highlight the need for targeted treatment in this rare tumor type and a prospective study is needed to formally assess the response rate and clinical benefit.

INTRODUCTION

Epithelial ovarian cancer (EOC) is a heterogeneous disease comprising several histological and molecular subtypes, and emerging molecular analyses are challenging longstanding clinical treatment paradigms. EOC subtypes are characterised by different gene expression and somatic mutation patterns and varying degrees of sensitivity to current standard carboplatin/paclitaxel combination chemotherapy.[1-3] The predominant type of EOC is serous carcinoma, accounting for approximately 80% of cases. Serous carcinoma is further classified into two main subtypes: the more common and better-characterised high-grade serous carcinoma (HGSC), and the less common low-grade serous carcinoma (LGSC). LGSC is not generally responsive to standard platinum-based chemotherapy,[4,5] and outcome is poor in women with residual disease following debulking surgery,[6,7] underscoring the need for alternative therapeutic options.

LGSC is molecularly distinct from HGSC and is characterised by activating mutations of the RAS-MAPK pathway, displays few genomic changes and is typically *TP53* wild-type.[8-11] Oncogenic *BRAF* mutations, such as V600E, lead to constitutive activation of the MAPK pathway and can be found in several cancer types, most commonly in melanoma.[12] The reported frequency of *BRAF* mutations in LGSC varies from 2% to 33% [10,13-15] and they are also found in up to 46% of serous borderline tumors.[16,17]

Clinical trials have shown impressive response rates to BRAF inhibitors in *BRAF* mutant melanoma, and their use in metastatic melanoma is now considered standard of care.[18,19] Responses in other tumor types, including gastrointestinal stromal tumor, thyroid papillary cancer, hairy cell leukaemia and high-grade colorectal neuroendocrine tumors have also been reported.[20-25] However, some cancers types, such as colorectal adenocarcinoma have much lower response rates to BRAF inhibitors despite having the same somatic *BRAF* mutations.[26-28] Therefore, while oncogenic *BRAF* represents a potential therapeutic target, responses vary according to tumor type and clinical benefit is not always achieved. This has led to large

'basket' trials assessing response to BRAF inhibitors in multiple *BRAF* mutant cancers.[29] However definitive conclusions from assessment of response in rare tumor types remains difficult due to small patient numbers.

We present the characterisation of *BRAF* mutations in LGSC patients, including two patients with $BRAF^{V600E}$ mutation who demonstrated substantial clinical, biochemical and radiological responses following treatment with BRAF inhibitors.

METHODS

The study population consisted of women diagnosed with LGSC between 1992 and 2015 (n=65). The women were identified in the Australian Ovarian Cancer Study (AOCS, http://www.aocstudy.org) or the Gynaecological Oncology Biobank at Westmead Hospital (GynBiobank) (Sydney, NSW). We incorporated the shift from a three tier grading system to a two tier grading system, as recommended in the 2014 World Health Organization (WHO) classification of ovarian tumors.[30] LGSC cases were identified from review of diagnostic pathology reports and independent review of hematoxylin and eosin (H&E) stained diagnostic slides by expert Gynae-pathologists. In addition, grade 2 cases were screened for *TP53* mutations and only those found to be wildtype, consistent with a molecular classification of LGSC, were included in the LGSC cohort.

Clinical Definitions.

Progression-free survival (PFS) was calculated as the time interval from date of histological or cytological diagnosis to the date of first progression based on Gynecological Cancer Inter Group (GCIG) criteria.[31] Overall survival (OS) was calculated from date of diagnosis to date of death from any cause. Treatment response was assigned according to GCIG CA125 criteria.[31] Briefly, ≥50% reduction in serum CA125 from an elevated pretreatment level, confirmed and maintained for at least 28 days was considered a response. The reverse Kaplan-Meier method was used to quantify follow-up time. [32]

Study Oversight.

Women recruited to AOCS and the GynBiobank provided written consent. AOCS was approved by the Human Research Ethics Committee at the Peter MacCallum Cancer Centre, Queensland Institute of Medical Research, Westmead Hospital and all other participating hospitals. The GynBiobank and this study were approved by the Western Sydney Local Health District Human Research Ethics Committee.

Written informed consent was additionally obtained from the patient treated with dabrafenib to include clinical information and imaging, also approved by the Western Sydney Local Health District Human Research Ethics Committee.

Sequencing and Immunohistochemistry.

Frozen or fixed tumor samples were sectioned, H&E stains were used to assess tumor content before and after serial sectioning for nucleic acid extraction. For samples containing >70% tumor, 1 x 100 μm section were used for DNA extractions. For samples containing <70% tumor, needle dissection of tumor cells was performed on up to 50 x 10 μm sections. Extractions were performed using the DNeasy Blood and Tissue kit or QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA was quantified using the Qubit® dsDNA BR assay (Invitrogen, Carlsbad, CA, USA).

Targeted multigene sequencing. Mutations in exon 15 of *BRAF*, exon 2 of *KRAS,* exon 20 of *ERBB2* and exons 2-11 of *TP53* were screened using high-resolution melt (HRM) analysis and validated by direct sequencing as previously described.[8,33] A subset of cases was screened by next-generation sequencing. Target enrichment of the DNA samples was performed according to the manufacture's protocol (Qiagen GR DNAseq Targeted Panels V2 Handbook 06/2015 protocol; NGHS-006x Human Ovarian Cancer Panel). The PCR reactions for each sample were pooled and target enriched DNA were purified using Agencourt AMPure XP beads (Beckman Coulter, Indianopolis, Indiana, USA). The DNA Libraries were prepared following Qiagen

QIAseq 1-Step Amplicon Library Preparation Handbook 01/2016. DNA libraries (> 4 nM) were sequenced on a MiSeq (Illumina, Australian Genome Research Facility, Melbourne) read length 2 x150 bp. Sequencing coverage read depth of 1200x was achieved for each sample.

Exome and whole genome sequencing. Whole genome and exome sequencing data and analysis was obtained for 22 cases using snap frozen tumor tissue and matched normal DNA from peripheral blood lymphocytes as previously described.[33,34]

Immunohistochemistry. Samples of the ovarian tumor from the primary surgery specimen and in biopsies at disease relapse, were stained for mutated $BRAF^{V600E}$ using a mutation specific monoclonal antibody (mouse anti-human $BRAF^{V600E}$ monoclonal antibody (clone VE1), Spring Biosciences, Pleasanton, CA, USA) using the Ventana BenchMark ULTRA IHC staining module with a 1 in 200 antibody dilution and reviewed by a pathologist (RB).

RESULTS

Frequency of *BRAF* mutations in LGSC

BRAF-mutation-positive cases were identified among a LGSC cohort of 65 patients (WES/WGS, n = 22; targeted multigene sequencing, n = 43). Molecular characterisation revealed 9/65 (13.8%) to have a *BRAF* mutation. Representative photomicrographs of the *BRAF*-mutation positive cases are shown in Figure 1. Most were $BRAF^{V600E}$ (8/9, 89%), a 'hot-spot' mutation locus in diverse cancer types, and one had a $BRAF^{L597R}$ mutation, an uncommon missense variant (Table 1).

In three *BRAF* mutant cases whole exome and whole genome sequencing additional variants predicted to alter protein sequence (ranging from 13 to 30 per case, Table 2) at lower allele frequencies compared with the *BRAF* mutations, suggestive of sub-clonal events.[33] There was no evidence of additional driver mutations and we found no genes with deleterious mutations that were common to the three cases, apart from *BRAF*.

Clinical features of *BRAF*-mutation positive LGSC cases

Among *BRAF*-mutation positive LGSC cases, patient age at diagnosis ranged from 22 to 77 years (median 51 years) (Table 1). The majority had FIGO stage III or IV disease at diagnosis (7/9, 78% patients).

All patients with *BRAF*-mutated LGSC had surgery as part of primary treatment and most were optimally debulked to no macroscopic residual disease (7/9, 78%). The median follow-up time was 61.5 months and five of the nine cases (56%) have remained progression-free (Table 1).

Treatment and clinical course in patients with *BRAF*-mutation positive LGSC following disease progression

Four patients progressed and all had a relatively short PFS (Table 1, Figure 2). None of these four patients responded to chemotherapy in the relapsed setting. The first two patients, Case 6 and Case 8 (Table 1) were treated with conventional chemotherapy and had poor overall survival (Figure 2A and Figure 2B), in contrast with two patients who received targeted treatment (Figure 2C and Figure 2D).

Case 6 was a 51-year-old woman diagnosed with stage IIIC LGSC, who was optimally debulked to no residual macroscopic disease (Figure 2A). The patient progressed within 5 months of completing primary carboplatin and paclitaxel chemotherapy, and was commenced on second line chemotherapy with pegylated liposomal doxorubicin (Caelyx). There was no response by CA125 criteria[31] and the patient subsequently received third line etoposide chemotherapy but progressed and died two months after the completion of treatment, resulting in an overall survival of less than two years from her initial diagnosis.

Case 8 was diagnosed with stage IV ovarian cancer at age 31 (Figure 2B). She received 3 cycles of neoadjuvant chemotherapy (carboplatin / paclitaxel) with no significant response by CA125 criteria. She proceeded to surgery and was debulked to ≤ 1 cm residual disease, with pathology confirming Grade 1 serous carcinoma arising from borderline serous cystadenoma. After a further 5 cycles of carboplatin and paclitaxel her CA125 decreased (51 U/ml) but did not

normalise. Disease progression was evident within two months of completing chemotherapy, with CA125 increasing to 502 U/ml, and the patient died a month later, less than a year from diagnosis.

Case 4 was a 22-year-old who presented with abdominal pain. Pelvic ultrasound revealed a complex ovarian mass and serum CA125 was significantly elevated (647 U/mL; normal range <35 U/mL). At surgery the patient was found to have widespread peritoneal disease and histopathology revealed a serous borderline tumor with invasive implants throughout the peritoneum, FIGO stage IIIB. She was debulked to no macroscopic residual disease and despite six cycles of chemotherapy with carboplatin and paclitaxel, her CA125 level remained elevated (38 U/mL) at the end of primary treatment (Figure 2C), indicative of chemo-resistant residual disease.

Eleven months later imaging confirmed progressive LGSC resulting in secondary debulking surgery and further chemotherapy. At further progression, the patient was entered into a phase I trial of the BRAF inhibitor dabrafenib[20] (GlaxoSmithKline, Australia) and received 100 mg bi-daily (bd). Her best RECIST 1.1[35] response during the trial was stable disease with a 28% decrease of the target lesion. During the study, an interruption in dabrafenib for toxicity, resulted in a CA125 spike to 238 U/ml, which promptly decreased following resumption of dabrafenib. After 10 months of dabrafenib therapy, the patient came off study due to a combination of toxicity and progressive disease.

With subsequent further disease progression, the patient received chemotherapy with carboplatin/gemcitabine and liposomal doxorubicin but there was no response by CA125 criteria.

At the time of disease progression following chemotherapy, biopsy of supraclavicular and para-aortic lymph node confirmed the presence of $BRAF^{V600E}$ mutation by immunohistochemistry (Figure 3B, 3C), and she was recommenced on dabrafenib via a compassionate access scheme.

On therapy with dabrafenib 150 mg bd, (50% higher than her previous dose), there was an impressive clinical response, with reduced analgesic requirements, improved well-being and marked reduction in the palpable SCF lymph node. After seven months of therapy, her CA125 fell to within the normal range, for the first time since diagnosis. A PET/CT scan confirmed a significant partial radiological response to treatment (63% decrease in sum of diameters of measured lesions) and significantly less metabolically active lesions throughout almost all nodal regions and pulmonary nodules (Figure 4).

At the time of censoring for this report the patient was still on dabrafenib and the CA125 had remained within normal range for 4 months, confirming a complete CA125 response according to GCIG CA125 criteria.[31]

The final patient, Case 9, was a 71-year-old woman who was diagnosed with stage IV disease at diagnosis with a pleural effusion and CA125 was elevated at 275 U/ml (normal <36 U/ml). She received one cycle of neoadjuvant carboplatin and paclitaxel prior to being admitted with a bowel obstruction and undergoing debulking surgery. Histopathology confirmed LGSC and mutation testing revealed a *BRAF*^{V600E} mutation. On progression, she was enrolled into a BRAF inhibitor basket trial (manuscript in preparation) and has been on treatment for over 12 months. This patient had a partial radiological response (personal communication, Bo Gao) and normalization of her CA125 on treatment with a BRAF inhibitor (Figure 2D).

DISCUSSION

We report here clinical outcomes in one of the largest series of patients with *BRAF*-mutation positive LGSC reported to date and, for the first time to our knowledge, report response to the BRAF inhibitor, dabrafenib, in a patient with LGSC with a somatic *BRAF*^{V600E} mutation.

LGSC is typically *TP53* wild-type and commonly harbors RAS and RAF pathway mutations.[8,13,17] This finding has led to clinical trials evaluating MEK inhibitor activity. A phase 2 trial evaluating response to MEK inhibitor, selumetinib in LGSC reported a promising

response rate of 15%.[36] Response was not associated with known *BRAF/KRAS* mutation status, although this may be due to the small number of cases with known mutations in the trial. It is not clear whether all mutation subtypes of LGSC respond similarly to MEK inhibition.

We found *BRAF* mutations in 13.8% (9/65) of LGSC, which falls within the broad range reported previously (2% - 33%).[10,13-15,37] This was similar to 17.9% (10/56) reported by Xing *et al*[37] in a similar sized cohort and slightly higher than AACR GENIE Project database, where the frequency of patients with BRAF mutations in the LGSC was 4/56 (7%, accessed April, 2017).[38] The GENIE Project contains genomic records generated in CLIA-/ISO-certified laboratories obtained at multiple tertiary referral centers[38], and may be enriched for patients with late stage disease seeking biomarker-driven clinical trials, whereas the patients reported in the current study are prospectively recruited clinic-based cases.

Some studies have suggested that $BRAF^{V600E}$ mutations in LGSC are rare and associated with early stage disease and improved prognosis.[14,15,39] However, we found that most women with a *BRAF* mutation positive tumor were diagnosed at an advanced stage. In our cohort, most women were able to be debulked to no residual disease, which is associated with improved prognosis. However, in the relapse setting $BRAF^{V600E}$ mutation positive LGSC was not responsive to chemotherapy. Four of the nine BRAF mutation positive patients identified progressed soon after primary treatment. All responded poorly to chemotherapy, which is characteristic of LGSC.[4,5] Two received conventional treatment and died within two years of diagnosis. Two received BRAF inhibitors and both achieved sustained response.

Case 4 had initially received dabrafenib as part of a dose-finding phase I trial[20] and while some response was seen, indicated by deceased serum CA125 levels, a much more profound response was seen when the patient was re-treated with dabrafenib 46 months later. The explanation for improved response with subsequent dabrafenib treatment is potentially multifactorial and may include the increased dosage, in line with current melanoma dosing guidelines, possibly

suggesting that her original dose was sub-therapeutic.[40] The patient also had a 25 kg weight loss between dabrafenib treatment periods, although there is no evidence to date that dabrafenib dose requires adjustment for weight.[40] There is also emerging evidence that BRAF inhibitors may act in part via an effect on host immunity[41,42], and it is possible that an uncharacterised immunological component contributed to response in this patient.

Consistent with our findings, responses to another BRAF inhibitor vemurafenib, have been reported in LGSC. Combe *et al* reported a response in a similarly heavily pre-treated woman who achieved a durable partial response to vemurafenib according to RECIST and CA125 criteria for over 21 months[43]. Similarly, in a large basket trial of vemurafenib in multiple cancer types, the one patient with LGSC also showed a sustained (over 12 months) partial response to vemurafenib,[29] Case 9 was also the only LGSC patient in a basket trial of a novel BRAF inhibitor (manuscript in preparation) and has shown a sustained response, providing additional evidence that LGSC patients with *BRAF*^{V600E} mutation-positive tumors are broadly responsive to BRAF inhibition.

In conclusion, recurrent LGSC is relatively chemotherapy resistant and targeted treatment may play an important role in improving patient outcome. Our results are consistent with recent reports of response to BRAF inhibition in at least two other studies [29,43] and suggest that BRAF inhibitors may be an effective option in patients with relapsed, *BRAF*-mutation positive LGSC. Moreover, the frequency in which these mutations were detected, indicates the importance of routine molecular testing for *BRAF*^{V600E} mutations in all advanced LGSC. The results also highlight the need for novel clinical trial design, such as platform trials, as traditional clinical trials are unlikely to be effective in identifying effective treatments for rare ovarian cancer subtypes.

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FIGURE LEGENDS

Figure 1. Representative histological sections of *BRAF*-mutated LGSC cases. Sections (4 μm) from formalin fixed, paraffin embedded tumor tissue blocks were stained with Hematoxylin and Eosin. (A) Case 1, Publishing ID 65928; (B) Case 2, Publishing ID 11368; (C) Case 3, Publishing ID 5711; (D) Case 4, Publishing ID 65917; (E) Case 5, Publishing ID 65854; (F) Case 6, Publishing ID 10693; (G) Case 7, Publishing ID 9125; (H) Case 8, Publishing ID 11014; (I) Case 9, Publishing ID 66198.

Figure 2. The serum CA125 levels and treatment throughout the clinical course of patient (A) Case 6, Publishing ID 10693; (B) Case 8, Publishing ID 11014; (C) Case 4, Publishing ID 65917 and (D) Case 9, Publishing ID 66198.

Figure 3. Expression of $BRAF^{V600E}$ in Case 4. Immunohistochemical staining using an antibody specific for $BRAF^{V600E}$ in formalin fixed, paraffin embedded tumor tissue sections of (A) Left ovarian tumor from the primary surgical specimen (B) Left supraclavicular fossa lymph node core biopsy prior to second dabrafenib treatment at 78 months (C) Left para-aortic lymph node core biopsy prior to second dabrafenib treatment at 78 months. The bar indicates 50 μm scale.

Figure 4. PET images of Case 4. 18-F-FDG PET/CT (Siemens Biograph mCT) images prior to (A, B and C) and after 7 months of second dabrafenib treatment (D, E and F) indicating response to treatment with decrease in size and metabolic activity of lesions. The green arrows indicate physiological uptake in heart, kidneys and bladder.

The axial images of the left medial supraclavicular and deep paravertebral lesions prior to (B) and after 7 months of second treatment with dabrafenib (E). The right common iliac node prior to (C) and after 7 months of second treatment with dabrafenib (F).

Table 1: Clinico-pathological characteristics of *BRAF*-mutation positive low-grade serous ovarian cancer cases

Case	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Summary
Publishing ID	65928	11368	5711	65917	65854	10693	9125	11014	66198	n = 9
Age at diagnosis (years)	45	77	68	22	40	51	52	31	73	Median 51, Range 22 - 77
FIGO Stage	IA	IA	IIIA	IIIB	IIIC	IIIC	IIIC	IV	IV	I 2/9 (22%) III 5/9 (56%) IV 2/9 (22%)
Mutation	*BRAF*^{V600E}	*BRAF*^{V600E}	*BRAF*^{V600E}	*BRAF*^{V600E}	*BRAF*^{V600E}	*BRAF*^{L597R}	*BRAF*^{V600E}	*BRAF*^{V600E}	*BRAF*^{V600E}	*BRAF*^{V600E} 8/9 (89%)
Method	TS[a,b]	WES[b]	WES[b]	WES[b]	TS[b]	WGS[b]	TS[c]	TS	TS	*BRAF*^{L597R} 1/9 (11%)
Preoperative CA 125 (U/ml)	325	1824	1000	647	161	140	309	245	275	Mean 547; Range 140 - 1824
Normal range (U/ml)	<35	<35	<24	<35	<35	<30	<35	<35	<36	NB: different upper limit of normal
Residual disease after cytoreductive surgery	Nil	Nil	Nil	Nil	Nil	Nil	Nil	≤1 cm	>2 cm	No residual disease, 7/9 (78%)
Primary chemotherapy (carboplatin/paclitaxel)	No	No	No	Yes	Yes	Yes	Yes	Yes[e]	Yes[e]	Primary chemotherapy 6/9 (67%)
Progression	No	No[d]	No	Yes	No	Yes	No	Yes	Yes	Progression-free, 5/9 (56%)
Progression-free survival (months)	36.9	>38[d]	11.9	13.8	61.5	8.28	77.1	7.8	1	
Alive	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes	Alive, 6/9 (67%)
Overall survival (months)	36.9	68	11.9	78.6	61.5	23	77.1	8.9	24	

[a]TS (targeted sequencing), WES (whole exome sequencing), WGS (whole genome sequencing)

[b]Etemadmoghadam *et al,* 2017, [c] Emmanuel *et al,* 2014,

[d] Lost to follow-up after 38 months (ie no progression information between 38 months and death at 68 months (cause of death not known)).

[e]Received neoadjuvant chemotherapy

Table 2: Summary of single nucleotide variants (SNVs) and insertions or deletions (indels) by sample

Sample	Mutation analysis[1]	High confidence mutations	Deleterious consequence	Driver gene (coding mutation; amino acid change)	Genes with deleterious mutation - no evidence of mutational cancer driver[2]
11368	WES	110	30	*BRAF* (c.1799T>A; p.V600E)	*ATP2B4, CCHCR1, CD163, CTD-2132N18.3, DCAF8L2, DCHS1, DOC2A, EFHC1, FFAR1, GALNT3, GTF3C2, HLA-C, IFFO1, ITGAV, KAT2A, LCA10, NPY5R, OAS1, PLEC, PRMT10, SECISBP2L, SETDB1, SHOC2, SLC15A1, SYNE2, TEX15, TTN, ZNF710, ZRANB2*
65917	WES	20	11	*BRAF* (c.1799T>A; p.V600E)	*BMP1, CA10, CACNA1C, CSMD1, DNM1, IFT172, KCNJ11, MAST2, OR11L1, STAT1*
10693 (AOCS-002)	WGS	25	13	*BRAF* (c.1790T>G; p.L597R)	*CC2D2B, CNGB3, DDHD2, ERN1, HPGD, NAPRT1, NPR3, PTPRB, RPGRIP1L, SYT14, TDO2, TLL2*

[1] Published in Etemadmoghadam *et al (2017)*

[2] Intergrative Oncogenomics (intOGen, https://www.intogen.org/search)