Impact of mutational status on survival in low-grade serous carcinoma of the ovary or peritoneum

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Background: Low-grade serous carcinoma of the ovary or peritoneum is a distinct, well-recognized histologic subtype characterized by young age at diagnosis, relative chemoresistance, and prolonged overall survival. Common mutations reported to be found within this subtype include KRAS and BRAF.

Methods: Using clinical information of patients from our IRB-approved registry and tissue from a subset of these patients, we performed mutational analysis for KRAS and BRAF using the direct Sanger sequencing technique and correlated findings with the clinical outcome, overall survival (OS).

Results: In 79 cases, patients with KRAS or BRAF mutations (n = 21) had a significantly better OS than those with wild-type KRAS or BRAF (n = 58) (106.7 months (95% CI, 50.6, 162.9) vs 66.8 months (95% CI, 43.6, 90.0)), respectively (P = 0.018).

Conclusions: Mutational status appears to be a potential prognostic factor in low-grade serous carcinoma of the ovary or peritoneum.

Low-grade serous carcinoma of the ovary or peritoneum is a distinct histologic subtype that may arise either de novo or following a diagnosis of serous tumour of low malignant potential (STLMP) (Crispens et al, 2002; Malpica et al, 2004; Gershenson et al, 2006; Shvartsman et al, 2007; Gershenson et al, 2015). Its clinical behaviour is characterised by young age at diagnosis, relative chemoresistance, and prolonged overall survival (OS) relative to high-grade subtypes of ovarian cancer (Gershenson et al, 2006; Gershenson et al, 2015). In addition, the diagnosis of the de novo presentation most commonly is made in the advanced stages.

In 2003, Singer and colleagues reported that KRAS and BRAF mutations occurred with a frequency of 35% and 33%, respectively, in low-grade serous carcinoma of the ovary, or what they termed, ‘invasive micropapillary serous carcinoma’ (Singer et al, 2003). However, subsequent reports indicated a much lower frequency of BRAF mutation in low-grade serous carcinoma (Wong et al, 2010; Grisham et al, 2012; Farley et al, 2013). The frequency of KRAS mutations ranged from 16 to 41% (Wong et al, 2010; Grisham et al, 2012; Farley et al, 2013). These results confirm that low-grade serous carcinoma has a distinct molecular pathway, and that, specifically, the mitogen-activated protein kinase pathway appears to have a major role in the pathogenesis of this subtype. Although the initial clinical trial of a MEK inhibitor, selumetinib, demonstrated promising activity in recurrent low-grade serous carcinoma of the ovary or peritoneum, with an objective response rate of 15%, there was no correlation between response and mutational status (Farley et al, 2013). Subsequent phase III clinical trials studying the activity and toxicity of different MEK inhibitors in recurrent low-grade serous carcinoma are ongoing; these trials include translational research objectives intended to re-test the hypothesis that response to this targeted therapy approach is correlated with activation of the mitogen-activated protein kinase pathway. The purpose of this study was to investigate OS based on KRAS or BRAF mutational status in low-grade serous carcinoma.
This study was approved by the institutional review board at the University of Texas M.D. Anderson Cancer Center. The Low-Grade Serous Tumor Database is a longitudinal database that contains clinico-demographic information from patients who have provided written informed consent in accordance with protocol guidelines. We identified patients with low-grade serous carcinoma of the ovary or peritoneum for whom tumour tissue was available for study. All patients provided written informed consent for use of their tumour specimens. Eligibility criteria for inclusion in this study were: (i) Original diagnosis of advanced stage STLMP with recurrence as metastatic low-grade serous carcinoma or de novo diagnosis of stage II–IV low-grade serous carcinoma; and (ii) adequate clinical information based on completeness of follow-up, date of last contact, and current status. Patients with STLMP without recurrence as low-grade serous carcinoma or those with stage I low-grade serous carcinoma were excluded. Pathology slides of all patients were reviewed by MD Anderson gynaecologic pathologists and documented as low-grade serous carcinoma using criteria that have been previously reported by our group (Malpica et al, 2004; Schmeler et al, 2011). Formalin-fixed, paraffin-embedded or frozen tissue blocks were retrieved from the Department of Pathology or the Gynecologic Oncology Tumor Repository, respectively, and MD Anderson gynaecologic pathologists confirmed tissue sections selected for mutational analysis for KRAS and BRAF to contain low-grade serous carcinoma. The primary site of disease was ovary for (17.7%) had metastatic tumour following an original diagnosis of 58 (82.3%) patients had stage II–IV de novo tumour, and 14 (17.7%) had metastatic tumour following an original diagnosis of de novo low-grade serous carcinoma. The primary site of disease was ovary for 13 (16.5%) patients. Median age at diagnosis was 46 years (range, 21–79 years). Most patients underwent surgery at some point during their clinical course, and most had multiple lines of systemic therapies. The majority of patients underwent primary cytoreductive surgery, had gross residual disease at completion of surgery, and initially received platinum-based chemotherapy. There were no significant differences in characteristics between wild-type and BRAF/KRAS mutation cases except for site (ovary vs peritoneum).

Mutational analysis revealed KRAS mutation (12 G12D, 2 G12V, 2 G12A, 1 G12S, and 1 G12R) in 18 (22.8%) cases and Braf V600E mutation in 3 (3.8%) cases, for a total of 21 mutations (26.6%). No detectable mutations (wild-type) of KRAS were identified in 58 (73.4%) cases. The median OS for LGSC contained a KRAS or BRAF mutation was 106.8 months (95% CI, 50.6, 162.9) compared with 66.8 months (95% CI, 43.6, 90.0) for the entire cohort of 79 patients was 81.3 months (95% CI, 66.1, 96.4 months). The median OS for women whose tumours contained a KRAS or BRAF mutation was 106.8 months (95% CI, 50.6, 162.9) compared with 66.8 months (95% CI, 43.6, 90.0)
for women whose tumours contained no KRAS or BRAF mutations ($P = 0.018$) (Figure 1).

The results of univariable and multivariable Cox proportional hazards regression are shown in Table 2. Only KRAS/BRAF mutation status, residual disease at the completion of surgery, and disease status at the completion of primary therapy were included in the multivariable analysis. Compared with the wild type, the presence of a KRAS/BRAF mutation conferred a protective effect on OS (HR = 0.49; 95% CI (0.26, 0.95); $P = 0.03$). Conversely, compared with no disease at the completion of primary therapy, the presence of persistent disease resulted in compromised OS (HR = 2.17; 95% CI (1.23, 3.83); $P = 0.007$).

**DISCUSSION**

Our preliminary results suggest that a KRAS or BRAF mutation may serve as a favourable prognostic factor and have a significant impact on outcome in women with metastatic low-grade serous carcinoma of the ovary or peritoneum. Furthermore, after adjusting for the effects of other variables, the influence of KRAS/BRAF mutational status on OS remained statistically significant. Potential limitations of this study include the traditional method of genomic sequencing and potential selection bias associated with patients seen in a tertiary care centre. In addition, we have combined cases of BRAF and KRAS mutations, not definitely understanding whether the effect of these mutations on

**Table 2. Univariable and multivariable Cox proportional hazards for overall survival**

| Variable                        | Univariable |                      |                  |                      |
|---------------------------------|-------------|----------------------|------------------|----------------------|
|                                 | Variable    | HR       | 95% CI         | $P$      | HR       | 95% CI         | $P$      |
| Primary site                    |             |          |                 |          |          |                 |          |
| Ovary (reference)               |             | 0.98     | 0.51, 1.88     | 0.95     |          |                 |          |
| Peritoneal                      |             |          |                 |          |          |                 |          |
| Age, years                      |             | 1.00     | 0.98, 1.02     | 0.81     |          |                 |          |
| Race                            |             |          |                 |          |          |                 |          |
| White (reference)               |             | 1.14     | 0.59, 2.26     | 0.69     |          |                 |          |
| Non-white                       |             |          |                 |          |          |                 |          |
| Surgery type                    |             |          |                 |          | 0.37     |                 |          |
| Primary CRS (reference)         |             | 0.84     | 0.41, 1.71     | 0.64     |          |                 |          |
| NACT followed by IDS            |             |          |                 |          |          |                 |          |
| No surgery                      |             | 0.38     | 0.09, 1.55     | 0.18     |          |                 |          |
| KRAS/BRAF mutation status       |             |          |                 |          |          |                 |          |
| Wild type (reference)           |             | 0.48     | 0.26, 0.89     | 0.02     | 0.49     | 0.26, 0.95     | 0.03     |
| Residual disease at completion of surgery | | 0.03 | | | 0.26 | | |
| No gross residual disease (reference) | | 2.41 | 1.26, 4.60 | 0.008 | | 1.53 | 0.74, 3.16 | 0.25 |
| Gross residual disease          |             |          |                 |          |          |                 |          |
| No surgery                      |             | 0.71     | 0.16, 3.20     | 0.65     | 0.46     | 0.10, 2.15     | 0.32     |
| Unknown                         |             | 1.57     | 0.50, 4.96     | 0.44     | 1.06     | 0.32, 3.51     | 0.92     |
| Disease status at completion of primary therapy | | 0.002 | | | 0.03 | | |
| No disease (reference)          |             | 2.46     | 1.44, 4.22     | 0.001    | 2.17     | 1.23, 3.83     | 0.007    |
| Disease present                 |             | 0.68     | 0.16, 2.89     | 0.60     | 1.03     | 0.23, 4.63     | 0.97     |
| No chemotherapy                 |             |          |                 |          |          |                 |          |
| Stage                           |             | 0.88     |                 |          |          |                 |          |
| II (reference)                  |             |          |                 |          |          |                 |          |
| III/IV                          |             | 1.31     | 0.41, 4.25     | 0.65     |          |                 |          |
| STLMP → LGSC                    |             | 1.21     | 0.39, 4.35     | 0.77     |          |                 |          |
| LGSC type                       |             |          |                 |          |          |                 |          |
| Recurrent LMP (reference)       |             | 1.07     | 0.57, 2.00     | 0.84     |          |                 |          |
| de novo LGSC                    |             |          |                 |          |          |                 |          |

Abbreviations: CI = confidence interval; CRS = cytoreductive surgery; HR = hazard ratio; IDS = interval debulking surgery; LGSC = low-grade serous carcinoma; NACT = neoadjuvant chemotherapy; STLMP = serous tumour of low malignant potential; STLMP → LGSC = serous tumour of low malignant potential → low-grade serous carcinoma; LMP = low malignant potential.
outcome is similar. We have done so because there are only three cases with BRAF mutation, but future studies of larger cohorts will hopefully further illuminate this issue. Future investigations need to include more sensitive next-generation sequencing techniques, interrogation of other gene mutations, such as NRAS, and a larger number of patients with comparable long follow-up times. For example, Emmanuel et al. (2014) found NRAS mutations in 9% of invasive serous carcinomas with adjacent STLMP. In addition, if our findings are confirmed, combined with future data on the activity of targeted therapies in low-grade serous carcinoma in the context of their molecular profile, this information may allow greater individualization of treatment.

In contrast to the findings of this study, several reports have suggested the association of KRAS or BRAF mutations with poorer outcome compared with wild-type KRAS or BRAF in a variety of malignancies (Andreyev et al., 2001; Souglakos et al., 2009; Johnson et al., 2012). The explanation for this potential discordance is unclear. The ability of oncogenes to induce senescence in normal cells and premalignant tumors is well established (Dhomen et al., 2009; Collado and Serrano, 2010; Vicent et al., 2010). In addition, when wild-type p53 is reactivated in a mouse hepatocellular carcinoma induced by oncogenic ras and knockdown of p53, tumours cells undergo senescence and activation of the immune system. In one report, immune cells rapidly cleared senescent tumour cells to prevent further progression or even resulted in regression (Xue et al., 2007).

As most low-grade ovarian serous cancer cells have wild-type p53, it is possible that this subtype with a KRAS mutation may have senescent tumour cells that are cleared by immune cells, thereby inhibiting tumour progression. However, further investigation to elucidate this potential mechanism is required.

Although low-grade serous carcinoma is associated with superior survival outcomes compared with high-grade serous carcinoma and other high-grade ovarian cancers, such as clear cell and high-grade endometrioid subtypes, nevertheless, over 70% of women with low-grade serous carcinoma relapse and ultimately succumb to their cancer. Thus, it is important that we continue to concentrate on better understanding the biology of this rare subtype while concomitantly working toward improving treatment.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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