(U. Bückner); Bonn (M. Wolfgarten); Braunschweig (R. Lorenz); Bremen (G. Doering, S. Feidicker); Chemnitz (P. Krabisch); Cuxhaven (U. Deichert); Deggendorf (D. Augustin); Dortmund (G. Kunz); Dresden (K. Kast); Düsseldorf (von Minckwitz G, C. Nestle-Krämling, M. Rezai); Ebersberg (C. Höß); Eggenfelden (J. Terhaag); Erlangen (P. Fasching); Eschweiler (P. Staib); Essen (B. Aktas); Esslingen (T. Kühn); Frankfurt am Main (F. Khandan, V. Möbus, C. Solbach, H. Tesch); Freiburg (E. Stickeler); Fürstenwalde (G. Heinrich); Fürth (H. Wagner); Gelsenkirchen (A. Abdallah); Gifhorn (T. Dewitz); Göttingen (G. Emons); Greifswald (A. Belau); Hagen (V. Rethwisch); Halle an der Saale (T. Lantzsch, C. Thomssen); Hamburg (U. Mattner, A. Nugent, V. Müller); Hameln (T. Noesselt); Hamm (F. Holms); Hanau (T. Müller); Hannover (J.-U. Deuker, I. Schrader); Herne (D. Strumberg); Hildesheim (C. Uleer); Homburg/Saar (E. Solomayer); Jena (I. Runnebaum); Kaiserslautern (H. Link); Karlsruhe (O. Tomé, H.-U. Ulmer); Kassel (B. Conrad, G. Feisel-Schwickardi); Kiel (H. Eidtmann); Köln (C. Schumacher, T. Steinmetz); Landshut (I. Bauerfeind); Lebach (S. Kremers); Leipzig (D. Langanke); Lich (U. Kullmer); Limburg (A. Ober); Lübeck (D. Fischer); Ludwigsfelde (A. Kohls); Ludwigshafen (W. Weikel); Magdeburg (J. Bischoff, K. Freese); Mainz (M. Schmidt, W. Wiest); Mannheim (M. Sütterlin); Marktredwitz (M. Dietrich) Minden (M. Grießhammer); München (D.-M. Burgmann, C. Hanusch, B. Rack, C. Salat, D. Sattler); Münster (J. Tio); Mutlangen (E. von Abel); Neuruppin (B. Christensen); Neuss (U. Burkamp); Oldenburg (C.-H. Köhne); Paderborn (W. Meinerz); Quedlinburg (S.-T. Graßhoff); Ravensburg (T. Decker); Recklinghausen (F. Overkamp); Reutlingen (I. Thalmann); Rheinfelden (A. Sallmann); Rosenheim (T. Beck); Rostock (T. Reimer); Rottweil (G. Bartzke); Saarbrücken (M. Deryal); Schweinfurt (M. Weigel); St Gallen, CH (J. Huober, P. Weder); Stade (C.-C. Steffens); Stadthagen (S. Lemster); Stendal (A. Stefek); Stralsund (F. Ruhland); Stuttgart (M. Hofmann, J. Schuster, W. Simon); Traunstein (U. Kronawitter); Trier (M. Clemens); Tübingen (T. Fehm); Ulm (W. Janni); Unna (K. Latos); Villingen-Schwenningen (W. Bauer); Weiden (A. Roßmann); Weinheim (L. Bauer); Weißenfels (D. Lampe); Wiesbaden (V. Heyl, G. Hoffmann, F. Lorenz-Salehi); Witten (J. Hackmann); Würzburg (R. Schlag).

Annals of Oncology 25: 2372–2378, 2014
doi:10.1093/annonc/mdu461
Published online 3 October 2014

The performance of BRCA1 immunohistochemistry for detecting germline, somatic, and epigenetic *BRCA1* loss in high-grade serous ovarian cancer

J. L. Meisel[1,†], D. M. Hyman[1,2,8,†*], K. Garg[3], Q. Zhou[4], F. Dao[5], M. Bisogna[5], J. Gao[6], N. D. Schultz[6], R. N. Grisham[1,8], M. Phillips[5], A. Iasonos[4], N. D. Kauff[1,5,7,8], D. A. Levine[5,8], R. A. Soslow[3,8,‡] & D. R. Spriggs[1,2,8,‡]

[1]Gynecologic Medical Oncology Service; [2]Developmental Therapeutics, Department of Medicine; [3]Department of Pathology; [4]Department of Epidemiology and Biostatistics; [5]Gynecology Service, Department of Surgery; [6]Computational Biology Program; [7]Clinical Genetics Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, USA; [8]Weill Cornell Medical College, New York, USA

Received 21 April 2014; revised 8 July 2014, 8 August 2014 and 15 September 2014; accepted 18 September 2014

Background: BRCA1 expression can be lost by a variety of mechanisms including germline or somatic mutation and promotor hypermethylation. Given the potential importance of *BRCA1* loss as a predictive and prognostic biomarker in high-grade serous ovarian cancer, we sought to evaluate the utility of BRCA1 immunohistochemistry (IHC) in screening for *BRCA1* loss by germline, somatic, and epigenetic mechanisms.

Patients and methods: Patients with advanced high-grade serous ovarian cancer who had previously undergone germline *BRCA1* testing were identified. Samples from each tumor were stained for BRCA1 and reviewed independently by two pathologists blinded to BRCA status. Tumors with abnormal BRCA1 IHC and wild-type germline testing underwent further evaluation for somatic *BRCA1* mutations and promoter hypermethylation. McNemar's test was used to

*Correspondence to: Dr David M. Hyman, Developmental Therapeutics, Department of Medicine, Memorial Sloan Kettering Cancer Center, 300 East 66th Street, Room 1353, New York, NY 10065, USA. Tel: +1-646-888-4544; Fax: +1-646-888-4546; E-mail: hymand@mskcc.org

†Both authors contributed equally.
‡Both authors contributed equally.

determine the association of BRCA1 IHC with germline *BRCA1* mutations and *BRCA1* loss through any mechanism. Kaplan–Meier methods were used to estimate overall survival (OS), and the log-rank test was used to assess differences between groups.

Results: Inter-rater reliability between the two pathologists on BRCA IHC interpretation was very good (kappa coefficient 0.865, $P = 0.16$; McNemar's test). BRCA1 IHC was abnormal in 36% (48/135) of cases. When compared with germline *BRCA1* status, BRCA1 IHC had a high negative predictive value (95.4%) but a low positive predictive value (PPV, 52.1%). When accounting for promoter hypermethylation and somatic mutations as alternative methods of *BRCA1* loss, the PPV rose to 87.5%. Five-year OS rate was 49.6% [95% confidence interval (CI) 26.3% to 69.3%] for patients with germline *BRCA1* mutations, 50.4% (95% CI 27.5% to 69.5%) for germline wild-type *BRCA1* and abnormal IHC, and 52.1% (95% CI 38.4% to 64.2%) for germline wild-type *BRCA1* and normal IHC ($P = 0.92$).

Conclusions: BRCA1 IHC interpretation was a highly reproducible and accurate modality for detecting germline, somatic, or epigenetic mechanisms of *BRCA1* loss. These results support further development of BRCA1 IHC as a potential biomarker for *BRCA1* loss in high-grade serous ovarian cancer.

Key words: *BRCA1*, immunohistochemistry, genetic testing, ovarian cancer, biomarkers, hypermethylation

introduction

BRCA1 germline mutations confer an improved prognosis in high-grade serous ovarian cancer through a variety of mechanisms [1, 2]. The absence of intact homologous recombination DNA repair due to loss of BRCA1 function renders these cancers sensitive to agents that cause double-stranded DNA breaks, such as platinum compounds [3–5]. More recently, poly(ADP) ribose polymerase (PARP) inhibitors have also shown promising activity in BRCA1 germline mutant tumors [6, 7]. It is not known, however, whether other common mechanisms of *BRCA1* loss, including somatic mutation or promoter hypermethylation [8], confer a similarly favorable prognosis or improved sensitivity to DNA damaging agents or PARP inhibitors.

The lack of widely available means of identifying *BRCA1* somatic mutations and promoter hypermethylation in the clinic has hampered the ability to understand the clinical significance of these alterations. Even as somatic tumor sequencing becomes more commonplace, predicting the functional impact of low-frequency mutations on BRCA1 protein expression can be challenging. A straightforward method to directly identify BRCA1 protein loss would be potentially useful in prognostication, stratification for clinical trials, and selection among standard and investigational therapies for recurrent ovarian cancer. To address this unmet need, we evaluated the utility of BRCA1 immunohistochemistry (IHC), an inexpensive and widely available technique, in screening for *BRCA1* loss by germline, somatic, and epigenetic mechanisms.

methods

patient selection

Institutional Review Board approval was obtained for this analysis. Eligible patients were seen at Memorial Sloan Kettering Cancer Center (MSK) between 1 August 1996 and 1 August 2010 for newly diagnosed stage III or IV high-grade serous ovarian, fallopian tube, or primary peritoneal cancer. All diagnoses were confirmed by two expert gynecologic pathologists. All patients had consented to *BRCA1* mutation testing on one of two IRB-approved studies being conducted by the Clinical Genetics Service [9]. Patients who did not have tumor specimens available at MSK for analysis were excluded. To minimize ascertainment bias, patients whose first visit to MSK occurred >6 months after their date of diagnosis were also excluded from the survival analysis. All *BRCA1* mutations were predicted to be deleterious. Patients with variants of unknown significance were considered to be *BRCA1* wild-type.

BRCA immunohistochemistry

A triplicate tissue microarray containing samples from each tumor was prepared and stained for BRCA1 using previously published methods [10]. MS110 from Calbiochem (EMD Millipore, Billerica, MA), Catalog number OP92, was used. The epitope for this antibody is 304 amino acids from the N-terminus of BRCA1. Each IHC stain was reviewed independently by two pathologists blinded to BRCA status of each tumor, and scored as absent, equivocal, or retained using the following criteria:

- Loss: <5% of tumor nuclei staining, positive internal control (Figure 1A).
- Equivocal: 5%–10% of tumor cell nuclei staining, less intense compared with the positive internal control (Figure 1B).
- Retained: >10% of tumor cell nuclei staining, positive internal control or >5% when staining intensity of tumor cell nuclei is similar to the internal control (Figure 1C).

The stromal cells serve as an internal positive control of the validity of the stain, since they retain a normal copy of *BRCA1* even if tumor cells do not. If BRCA1 was retained, no further sections were obtained. If BRCA1 was scored absent or equivocal, a whole section was prepared and a final score obtained using the same procedure. This was done to avoid false negatives associated with patchy BRCA1 IHC staining. Of note, even tumors with retained staining do not typically show diffuse staining of strong intensity; rather, staining is often of moderate to strong intensity and limited in distribution to 30%–60% of tumor cells, usually in a patchy rather than a geographic pattern.

Tumors with retained staining were considered 'normal' and those with absent or equivocal staining were considered 'abnormal'. To evaluate whether BRCA1 IHC may have utility as a dynamic biomarker, BRCA1 IHC stains were repeated in recurrent tissue (when available) from patients with abnormal BRCA1 IHC in pretreatment tissue and correlated to platinum sensitivity status.

BRCA1 somatic sequencing and promoter hypermethylation testing

Tumors with abnormal BRCA1 IHC and wild-type germline testing underwent further testing to assess for somatic *BRCA1* mutations and *BRCA1*

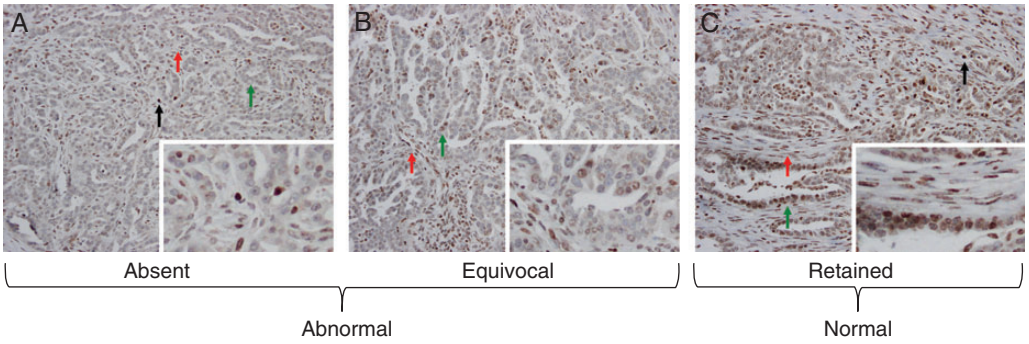

Figure 1. Representative BRCA1 IHC stains and Interpretation. Low and high power images of loss (A), equivocal (B), and retained (C) BRCA1 IHC stains based on percent of tumor nuclei staining (green arrow). Loss and equivocal samples were considered abnormal and retained samples normal. Stroma (red arrow) and tumor infiltrating lymphocytes (black arrow) were used as positive controls.

DNA promoter hypermethylation. DNA was extracted from microdissected formalin-fixed, paraffin-embedded tumor and normal tissues according to standard laboratory protocols. All tumors contained a minimum of 50% tumor cell nuclei. We used a custom target capture deep sequencing assay to perform massively parallel sequencing across the entire coding region of *BRCA1*. Paired normal and tumor samples were sequenced to a median depth of 272× with 96.5% of the targeted sequence in *BRCA1* covered at 100× or greater. Custom oligonucleotide probes were designed to capture all protein-coding exons and splice regions. Captured regions were sequenced on an Illumina HiSeq 2000 (Illumina, San Diego, CA) and reads were aligned to the reference human genome (hg19). All candidate mutations were manually reviewed using the Integrative Genomics Viewer [11].

A CLIA-approved pyrosequencing assay was used for the simultaneous analysis and quantification of the degree of methylation at 11 CpG sites in the established promoter region of BRCA1 [12]. Bisulfite treatment of genomic DNA samples was used in the hydrolytic deamination of non-methylated cytosines to uracils, whereas methylated cytosines are resistant to conversion. After a PCR, the methylation status at a given position is manifested in the ratio C (former methylated cytosine) to T (former nonmethylated cytosine) translating epigenetic information into sequence information and can be analyzed in the bisulfite-treated DNA. With each run of the BRCA1 methylation assay, positive, negative, and no template controls are included. The assay was performed once and not in replication. The degree of methylation is calculated as allele frequency using the following formula:

$$\text{methylation}\,\% = \frac{\text{peak height methylated}}{\text{peak height methylated} + \text{peak height nonmethylated}} \times 100$$

The average methylation fraction across 11 CpG sites is reported as positive if the mean methylation is between 10% and 99% and negative if it is <10%. The percent of methylation in normal and hypermethylated cases is provided in supplementary Figure S1, available at *Annals of Oncology* online.

statistical methods

McNemar's test was used to determine the association of BRCA1 IHC with *BRCA1* germline test results as well as the association of BRCA1 IHC with *BRCA1* loss through any mechanism. The agreement between pathologists was assessed via the kappa statistic. Overall survival (OS) was defined from the diagnosis date to the last follow-up date or the death date for all patients. Kaplan–Meier methods were used to estimate OS and the log-rank test was used to assess differences between patients (i) with germline *BRCA1* mutations and (ii) without germline mutations who had (i) normal and (ii) abnormal IHC. Variables were regarded as significant at a level of 0.05.

Table 1. Patient and disease characteristics ($N = 135$)

Characteristic	Number	Percent
Age at diagnosis		
Median (mean)	59 (57.69)	
Range	32–82	
Stage		
IIIB	5	3.7
IIIC	99	73.3
IV	31	23
Optimally debulked		
Yes	102	77.9
No	29	22.1
Intraperitoneal chemo		
Yes	60	47.2
No	67	52.8
BRCA1 germline		
Wild-type	106	78.5
Mutant	29	21.5
Tissue stained		
Primary tumor, chemo-naïve	103	76.3
Primary tumor, chemo-treated	17	12.6
Recurrent tumor	15	11.1
Tumor site stained		
Ovary/tube	114	84.4
Other	21	15.6
BRCA1 IHC result		
Abnormal	48	35.6
Normal	87	64.4

results

Patient and disease characteristics describing the 135 patients analyzed are reported in Table 1. All patients had stage III or IV high-grade serous disease. Just over 20% harbored *BRCA1* germline mutations. Approximately 90% of tumor specimens were from initial surgical resection. Of these cases, 14% had received neoadjuvant chemotherapy. The ovary or fallopian tube specimens were used in 84% of cases and a variety of metastatic sites for the remainder.

Inter-rater reliability between the two pathologists on final BRCA IHC interpretation was very good (kappa coefficient

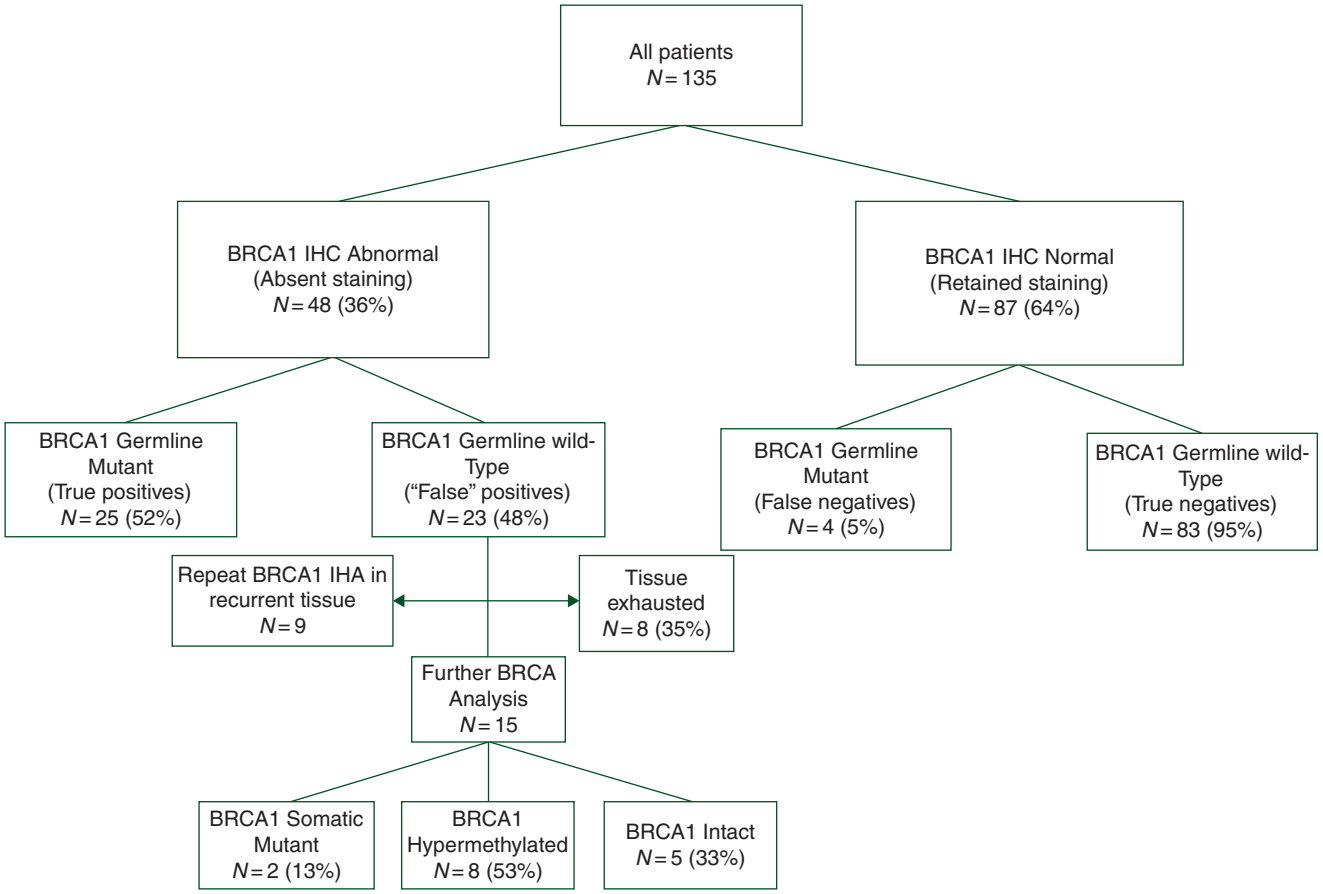

Figure 2. Consort diagram.

Table 2. Performance of BRCA IHC by mechanism of loss

	BRCA1 loss (germline only)		*BRCA1* loss (germline/somatic/methylation)	
	No	Yes	No	Yes
BRCA1 IHC results				
Normal	83	4	83	4
Abnormal	23	25	5	35
	Rate	95% CI	Rate	95% CI
BRCA1 IHC performance				
Sensitivity	86.2%	73.7% to 98.8%	89.7%	80.2% to 99.3%
Specificity	78.3	70.5% to 86.2%	94.3%	89.5% to 99.2%
PPV	52.1%	38.0% to 66.2%	87.5%	77.3% to 97.8%
NPV	95.4%	91.0% to 99.8%	95.4%	91.0% to 99.8%
OCCR	80.0%	73.3% to 86.8%	92.9%	88.5% to 97.4%

PPV, positive predictive value; NPV, negative predictive value; OCCR, overall correct classification rate.

0.865, $P = 0.16$; McNemar's test). The results of BRCA1 IHC testing are shown in Figure 2, and the performance of BRCA1 IHC testing with respect to *BRCA1* germline status and *BRCA1* loss by any mechanism is shown in Table 2. When compared with germline *BRCA1* status, *BRCA1* IHC had a high negative predictive value (95.4%) but a low positive predictive value (PPV, 52.1%). However, when accounting for promoter hypermethylation and somatic mutations as alternative mechanisms of *BRCA1* loss, the PPV of BRCA1 IHC rose to 87.5% and the overall correct classification rate was 92.7%. BRCA1 staining characteristics were similar in germline and somatic mutant patients.

Figure 3 depicts the result of BRCA1 IHC testing by location and mechanism of *BRCA1* loss. There was no observable

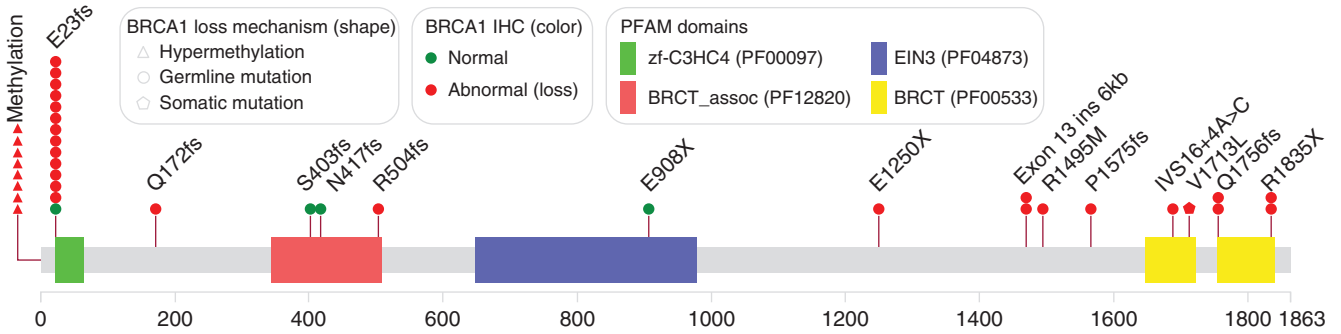

Figure 3. BRCA1 mutation and methylation map. E23fs is the 185delAG founder mutation. Q1756fs is the 5382/5385 insC founder mutation. One patient with multiple somatic BRCA1 mutations was not mapped.

Table 3. Overall survival (OS) by *BRCA1* status

BRCA1 status	*N*	Deaths	5-year OS rate (95% CI)	Median OS months (95% CI)	HR (95% CI)*
Germline mutant	28	14	49.6% (26.3% to 69.3%)	59.5 (48.2–95.0)	Ref. level
Germline WT, IHC abnormal	21	12	50.4% (27.5% to 69.5%)	64.8 (25.9–NE)	1.13 (0.52–2.46)
Germline WT, IHC normal	81	41	52.1% (38.4% to 64.2%)	65.9 (45.8–78.0)	1.13 (0.62–2.08)

*$P = 0.918$, obtained by using log-rank test.

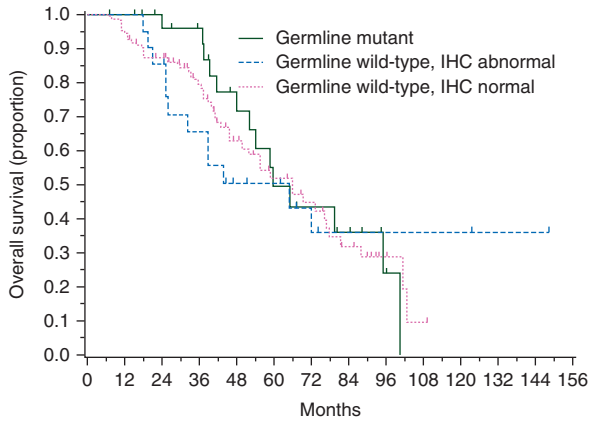

Figure 4. Overall survival by *BRCA1* status.

relationship between the location of *BRCA1* mutation and the BRCA IHC result. Recurrent tissue was available for nine germline wild-type patients who had abnormal BRCA IHC staining in the primary tumor specimen. Seventy-eight percent (7/9) had reverted to normal BRCA1 IHC staining in the recurrent specimen and 86% (6/7) of these cases were resistant to platinum therapy (platinum-free interval ≤12 months).

For the OS analysis, five patients were excluded because the interval from diagnosis to first evaluation at MSK was >6 months. The results for the OS analysis are shown in Table 3. Of the remaining 130 patients, 67 (51.5%) died of disease. The median duration of follow-up was 44.6 months (range: 7.2–148.3 months) for the 63 survivors. The 5-year OS rate was 49.6% [95% confidence interval (CI) 26.3% to 69.3%] for patients with germline *BRCA1* mutations, 50.4% (95% CI 27.5% to 69.5%) for germline wild-type *BRCA1* and abnormal IHC, and 52.1% (95% CI 38.4% to 64.2%) for germline wild-type *BRCA1* and normal IHC. Differences between the three groups did not reach statistical significance ($P = 0.92$). The Kaplan–Meier curves for OS, stratified by *BRCA1* germline and IHC status, are shown in Figure 4.

discussion

In this comprehensive study of BRCA1 immunohistochemical testing in high-grade, advanced-stage, ovarian serous carcinomas, we found that this is an effective method to identify *BRCA1* loss through both genetic and epigenetic mechanisms. When considering both mechanisms of *BRCA1* loss, BRCA1 IHC correlation was excellent, with an overall correct classification rate of 93%. Our findings are consistent with, and expand upon, previous smaller case series of BRCA1 IHC testing performed to date [10, 13–15].

At present, *BRCA1* germline testing is the only form of *BRCA1* assessment routinely offered to patients with ovarian cancer. BRCA1 IHC identified 86% of patients with a *BRCA1* germline mutation and therefore is not accurate enough to be used as prescreening before germline testing; however, our data indicate that in conjunction with routine *BRCA1* germline testing, BRCA IHC may provide a reliable means of identifying patients with nongermline mechanisms of *BRCA1* loss. As the clinical significance of somatic mutations and promoter hypermethylation is further ascertained, IHC could become useful as a companion to germline testing.

The results of BRCA1 staining were highly reproducible among pathologists blinded to the underlying *BRCA1* status of

each patient. Our two pathologists reached agreement in 126 of 134 (94%) cases. Moreover, in the eight cases where agreement was not reached, the disagreement was between absent versus equivocal staining. Therefore, none of the discrepancies involved scores that would have altered the final interpretation of the result (i.e. normal versus abnormal). Further studies will be necessary to determine whether similar inter-rater reliability would exist across institutions or among pathologists with less experience with BRCA1 IHC.

We did not find a difference in OS between *BRCA1* mutant, *BRCA1* wild-type/IHC normal, and *BRCA1* wild-type/IHC abnormal patients. Due to the small numbers of patients in each cohort and the convenience sample utilized, this analysis must be considered exploratory. The relatively small survival advantage conferred by *BRCA1* germline mutations necessitates a very large cohort study to detect a statistically significant difference compared with sporadic ovarian cancers [1]. This suggests that, if *BRCA1* somatic mutation or promoter hypermethylation conferred a similar survival advantage, a much larger population would be needed to detect this difference. Still, our results are consistent with a related analysis carried out and reported by The Cancer Genome Atlas [8]. Taken together, these data suggest that different mechanisms of *BRCA1* loss may be associated with unique disease phenotypes. Although other investigators have reported an association between BRCA1 protein expression and outcome in ovarian carcinoma, these reports have been somewhat limited by the absence of universal *BRCA1* germline testing and other assessments of *BRCA1* inactivation [16–18].

Unlike *BRCA1* germline sequencing, BRCA1 IHC may have utility as a dynamic biomarker throughout the disease course as methylation status changes or secondary gain-of-function mutations accumulate. Although our analysis was limited by the small number of recurrent samples, we observed that seven of eight patients—six of whom had developed at least intermediate resistance to platinum therapy—regained *BRCA1* function in recurrent specimens, consistent with preclinical observations of restoration of *BRCA1* function upon development of platinum resistance [3, 4]. This preliminary analysis suggests that BRCA1 IHC testing may be a useful biomarker for clinical trial selection or stratification, particularly in the setting of recurrent disease.

The importance of developing a real-time clinical test for nongermline mechanisms of *BRCA1* loss is heightened by the recent development of PARP inhibitors, which have been shown to produce objective responses [6, 7] and improved progression-free survival in *BRCA1/2* germline mutant ovarian cancers. However, the clinical benefit from PARP inhibitors is not limited to *BRCA1* or *BRCA2* germline mutated cancers. Recent investigations have identified deficiency in other key mediators of genomic stability including ATM [19], RAD51C [20], and MRE11 [21] that may also potentiate PARP inhibitor sensitivity. Importantly, preclinical data also indicate that *BRCA1* promoter hypermethylation [22] and somatic mutation [23] may also be synthetically lethal with PARP inhibitors. Taken together, these data suggest that BRCA1 IHC may be useful not only as a screen for *BRCA1* germline mutations but also as a predictive biomarker for PARP inhibitors in *BRCA1* germline wild-type patients.

conclusion

Detection of *BRCA1* loss in ovarian carcinomas has potential prognostic and therapeutic significance. Our data indicate that BRCA1 IHC might be an inexpensive, easy to implement, and reproducible means of identifying these patients in the clinic.

funding

Funded in part by the cancer center's core grant P30 CA008748. The core grant provides funding to institutional cores, such as Biostatistics and Pathology, which were used in this study. Also supported in part by a Stand Up to Cancer Dream Team Translational Research Grant, a Program of the Entertainment Industry Foundation (SU2C-AACR-DT0209), The Honorable Tina Brozman Foundation, and Be The Difference Foundation.

disclosure

The authors have declared no conflicts of interest.

references

1. Bolton KL, Chenevix-Trench G, Goh C et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA 2012; 307(4): 382–390.
2. Hyman DM, Zhou Q, Iasonos A et al. Improved survival for BRCA2-associated serous ovarian cancer compared with both BRCA-negative and BRCA1-associated serous ovarian cancer. Cancer 2012; 118(15): 3703–3709.
3. Husain A, He G, Venkatraman ES, Spriggs DR. BRCA1 up-regulation is associated with repair-mediated resistance to cis-diamminedichloroplatinum(II). Cancer Res 1998; 58(6): 1120–1123.
4. Sakai W, Swisher EM, Karlan BY et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature 2008; 451(7182): 1116–1120.
5. Gallagher DJ, Konner JA, Bell-McGuinn KM et al. Survival in epithelial ovarian cancer: a multivariate analysis incorporating BRCA mutation status and platinum sensitivity. Ann Oncol 2011; 22(5): 1127–1132.
6. Audeh MW, Carmichael J, Penson RT et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet 2010; 376(9737): 245–251.
7. Gelmon KA, Tischkowitz M, Mackay H et al. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. Lancet Oncol 2011; 12(9): 852–861.
8. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. Nature 2011; 474(7353): 609–615.
9. Scheuer L, Kauff N, Robson M et al. Outcome of preventive surgery and screening for breast and ovarian cancer in BRCA mutation carriers. J Clin Oncol 2002; 20(5): 1260–1268.
10. Garg K, Levine DA, Olvera N et al. BRCA1 immunohistochemistry in a molecularly characterized cohort of ovarian high-grade serous carcinomas. Am J Surg Pathol 2013; 37(1): 138–146.
11. Robinson JT, Thorvaldsdóttir H, Winckler W et al. Integrative genomics viewer. Nat Biotechnol 2011; 29: 24–26.
12. Esteller M, Silva JM, Dominguez G et al. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 2000; 92(7): 564–569.
13. Byrne TJ, Reece MT, Adams LA et al. An antibody assay predictive of BRCA1 mutations in ovarian tumors and normal tissue. Oncol Rep 2000; 7(5): 949–953.
14. Skytte AB, Waldstrom M, Rasmussen AA et al. Identification of BRCA1-deficient ovarian cancers. Acta Obstet Gynecol Scand 2011; 90(6): 593–599.

15. Vaz FH, Machado PM, Brandao RD et al. Familial breast/ovarian cancer and BRCA1/2 genetic screening: the role of immunohistochemistry as an additional method in the selection of patients. J Histochem Cytochem 2007; 55(11): 1105–1113.
16. Carser JE, Quinn JE, Michie CO et al. BRCA1 is both a prognostic and predictive biomarker of response to chemotherapy in sporadic epithelial ovarian cancer. Gynecol Oncol 2011; 123(3): 492–498.
17. Radosa MP, Hafner N, Camara O et al. Loss of BRCA1 protein expression as indicator of the BRCAness phenotype is associated with favorable overall survival after complete resection of sporadic ovarian cancer. Int J Gynecol Cancer 2011; 21(8): 1399–1406.
18. Weberpals JI, Tu D, Squire JA et al. Breast cancer 1 (BRCA1) protein expression as a prognostic marker in sporadic epithelial ovarian carcinoma: an NCIC CTG OV.16 correlative study. Ann Oncol 2011; 22(11): 2403–2410.
19. Gilardini Montani MS, Prodosmo A, Stagni V et al. ATM-depletion in breast cancer cells confers sensitivity to PARP inhibition. J Exp Clin Cancer Res 2013; 32: 95.
20. Min A, Im SA, Yoon YK et al. RAD51C-deficient cancer cells are highly sensitive to the PARP inhibitor olaparib. Mol Cancer Ther. 2013; 12: 865–877.
21. Koppensteiner R, Samartzis EP, Noske A et al. Effect of MRE11 loss on PARP-inhibitor sensitivity in endometrial cancer in vitro. PLoS One 2014; 9: e100041.
22. Ibragimova I, Cairns P. Assays for hypermethylation of the BRCA1 gene promoter in tumor cells to predict sensitivity to PARP-inhibitor therapy. Methods Mol Biol 2011; 780: 277–291.
23. Drew Y, Mulligan EA, Vong WT et al. Therapeutic potential of poly(ADP-ribose) polymerase inhibitor AG014699 in human cancers with mutated or methylated BRCA1 or BRCA2. J Natl Cancer Inst 2011; 103(4): 334–346.

Annals of Oncology 25: 2378–2385, 2014
doi:10.1093/annonc/mdu464
Published online 6 October 2014

Prognostic value of KRAS mutations in stage III colon cancer: *post hoc* analysis of the PETACC8 phase III trial dataset

H. Blons[1,2,3,†], J. F. Emile[4,5,†], K. Le Malicot[6,†], C. Julié[4], A. Zaanan[7], J. Tabernero[8], E. Mini[9], G. Folprecht[10], J. L. Van Laethem[11], J. Thaler[12], J. Bridgewater[13], L. Nørgård-Petersen[14], E. Van Cutsem[15], C. Lepage[16], M. A. Zawadi[17], R. Salazar[18], P. Laurent-Puig[1,2,3,†] & J. Taieb[7,2,†*]

[1]UMR-S1147, INSERM, Paris; [2]Paris Descartes University, Paris; [3]APHP Department of Biology, Georges Pompidou Hospital, Paris; [4]Department of Pathology, APHP Ambroise Paré Hospital, Boulogne-Billancourt; [5]Paris-Ouest University, Versailles Saint-Quentin; [6]Department of Statistics, Fédération Francophone de Cancérologie Digestive, Dijon; [7]APHP Department of Hepatogastroenterology and GI Oncology, Georges Pompidou Hospital, Paris, France; [8]Department of Medical Oncology, Vall d'Hebron University Hospital and Institute of Oncology (VHIO), Barcelona, Spain; [9]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [10]1st Medical Department, University Hospital Carl Gustav Carus, Dresden, Germany; [11]Deptartment of Gastroenterology, Erasme University Hospital, Brussels, Belgium; [12]Department of Internal Medicine IV, Klinikum Wels-Grieskirchen, Wels, Austria; [13]UCL Cancer Institute, University College London, London, UK; [14]Department of Oncology, Rigshospitalet, København, Denmark; [15]Department of Digestive Oncology, University Hospitals and KULeuven, Leuven, Belgium; [16]Department of Hepato-Gastroenterology, Dijon University Hospital and INSERM U 866, Dijon; [17]GI Oncology, Les Oudairies Hospital, La Roche sur Yon, France; [18]Catalan Institute of Oncology (IDIBELL), Barcelona, Spain

Received 10 July 2014; revised 18 September 2014; accepted 18 September 2014

Background: The prognostic value of *KRAS* mutations in colon adenocarcinoma is controversial. We examined this question as an ancillary study of the PETACC8 phase III trial.

Patients and methods: We analyzed the pronostic impact of *KRAS* exon 2 mutations in stage III colon cancer patients ($n = 1657$) receiving adjuvant FOLFOX ± cetuximab therapy included in the PETACC8 trial. Patients with *BRAF*-mutated cancers were excluded and, as no difference was found for time to recurrence (TTR) and disease-free survival (DFS) between treatment arms, both were pooled for analysis. Associations with TTR and DFS were analyzed using a Cox proportional hazards model.

Results: *KRAS* mutations were found in 638 of 1657 tumors and linked to shorter TTR ($P < 0.001$). However, when specific mutations were compared with wild-type, codon 12 mutations [hazard ratio (HR) 1.67, 95% confidence interval (CI) 1.35–2.04; $P < 0.001$] but not codon 13 (HR 1.23, 95% CI 0.85–1.79; $P = 0.26$) were significantly associated with shorter

*Correspondence to: Dr Julien Taieb, Georges Pompidou Hospital, Department of Hepatogastroenterology and GI Oncology, Université Paris Descartes, 20 rue Leblanc, 75015 Paris, France. Tel: +33-1-5609351; Fax: +33-1-56093441; E-mail: julien.taieb@egp.aphp.fr

†These authors contributed equally.