

# *BRAF* Mutations Occur Infrequently in Ovarian Cancer but Suggest Responsiveness to *BRAF* and MEK Inhibition

Maira P. Campos  
 Melissa Cohen  
 Erika Von Euw  
 Victor Velculescu  
 Jennifer L. Kujak  
 Dylan Conklin  
 Dorothy Hallberg  
 Dennis J. Slamon  
 Julia Elvin  
 Gottfried E. Konecny

Author affiliations and support information (if applicable) appear at the end of this article.

**Corresponding author:** Gottfried E. Konecny, MD, Division of Hematology-Oncology, Department of Medicine, University of California Los Angeles, 2825 Santa Monica Blvd, Ste 200, Santa Monica, CA 90404-2429; e-mail: gkonecny@mednet.ucla.edu.

Licensed under the Creative Commons Attribution 4.0 License



## INTRODUCTION

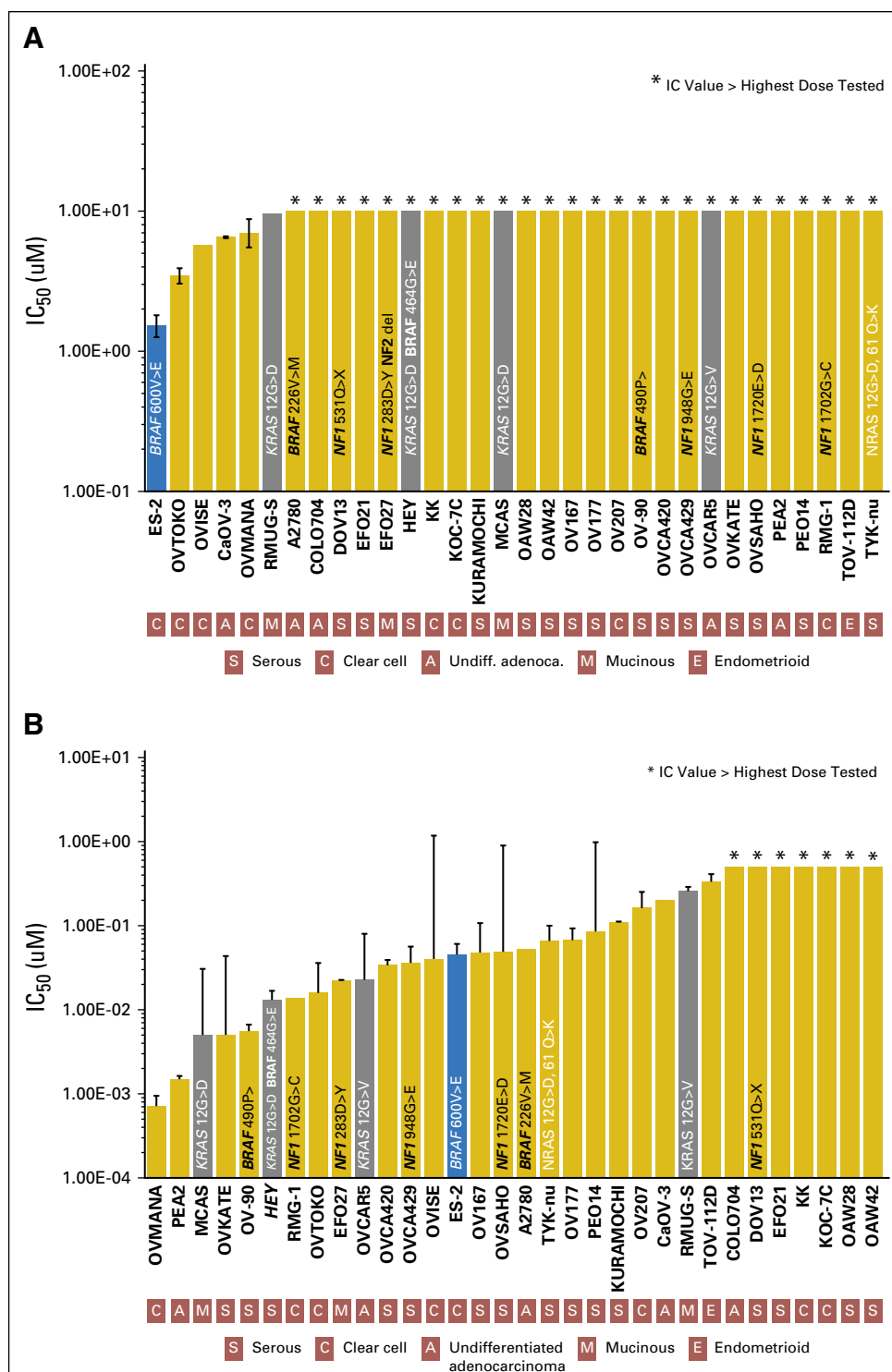
Ovarian cancer (OC) is the leading cause of death from gynecologic malignancies in the developed world.<sup>1</sup> Although substantial efforts have been devoted to identifying potentially actionable oncogenic driver mutations in OC, only a few genes are frequently mutated, particularly in high-grade serous OC (HGSOC), the most common histologic subtype. The Cancer Genome Atlas reported *p53* mutations in almost all primary HGSOC tumors (96%) but a low prevalence of recurrent somatic mutations in additional genes including *BRCA1* (12%), *BRCA2* (11%), *NF1* (4%), and *CDK12* (3%).<sup>2</sup> In the COSMIC database, additional infrequent mutations such as *KRAS* (6%), *PIK3CA* (2%), and *BRAF* (2%) have been identified in HGSOC.<sup>3</sup> We believe that some of these mutations, although rare, are important drivers in HGSOC. For example, mutations in *BRAF* lead to constitutive activation of downstream *MEK1* and *MEK2* and may regulate proliferation and survival in ovarian tumor cells as in many other cancers.<sup>4</sup> The most commonly observed *BRAF* mutation, V600E, accounts for 90% of the *BRAF* mutations found in all patients with cancer.<sup>3</sup> Treatment with *BRAF* inhibitors such as vemurafenib or dabrafenib in patients with advanced *BRAF*<sup>V600E</sup>-mutated melanoma has shown objective tumor responses in approximately half of the patients.<sup>5</sup> Recent clinical studies have suggested that concurrent inhibition of the *BRAF* and *MEK* kinases of the mitogen-activated protein kinase (MAPK) pathway with trametinib or cobimetinib can decrease MAPK-driven acquired resistance, resulting in greater efficacy and a decrease in the cutaneous toxicities observed from paradoxical MAPK pathway activation with *BRAF* inhibitor

monotherapy.<sup>6</sup> Here, we report a patient with recurrent HGSOC who had experienced treatment failure with an extensive number of prior treatment regimens and whose tumor was found to harbor a *BRAF*<sup>V600E</sup> mutation. Of note, this patient responded extremely well to treatment with a *BRAF* inhibitor (vemurafenib) and subsequently to a *BRAF* inhibitor in combination with a *MEK* inhibitor (dabrafenib and trametinib). To further underscore this treatment rationale, we studied the growth-inhibitory effects of the *BRAF* inhibitor vemurafenib and the *MEK* inhibitor trametinib across a panel of 32 OC cell lines that were each characterized for mutational status of *BRAF*, *KRAS*, and *NF1* (Fig 1). Cell lines, assays, and sequencing methods have been described earlier.<sup>7</sup> In brief, cells were plated into 24-well tissue culture plates and grown with or without increasing concentrations of inhibitors. Cells were counted on days 1 and 6 using a Coulter Z2 particle counter (Beckman Coulter, Indianapolis, IN). Growth inhibition was calculated as a function of the number of generations inhibited by vemurafenib or trametinib.

## INDEX PATIENT

A 70-year-old woman presented with recurrent *BRCA* wild-type HGSOC. Fourteen years before presentation, she had optimal cytoreductive surgery for International Federation of Gynecology and Obstetrics stage IIIC HGSOC followed by front-line chemotherapy with carboplatin plus gemcitabine and carboplatin plus paclitaxel. She developed recurrent disease to the left paracolic gutter region, which was not amenable to surgery but treated with carboplatin plus paclitaxel and, after further progression, by liposomal doxorubicin. Subsequent treatments included topotecan, pemetrexed, gemcitabine plus carboplatin,

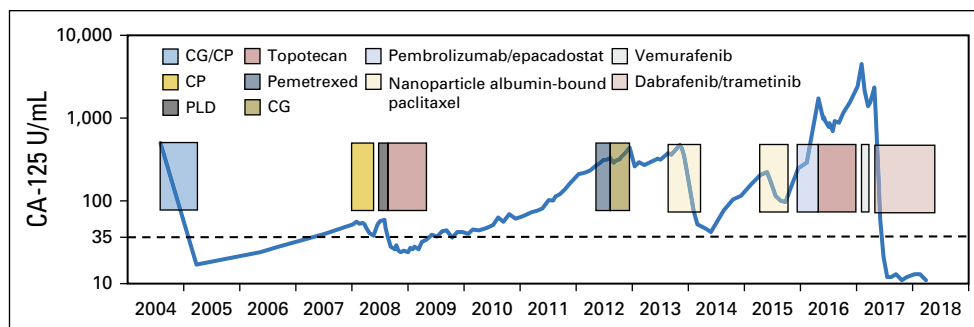
**Fig 1.** Preclinical activity of (A) vemurafenib and (B) trametinib across a large panel of 32 ovarian cancer (OC) cell lines characterized for *BRAF*, *KRAS*, and *NF1* mutations. Growth inhibition was calculated as a function of the number of generations inhibited in the presence of vemurafenib or trametinib versus the number of generations over the same time course in the absence of the drug as reported previously.<sup>7</sup> In brief, the log of the fractional growth inhibition was plotted against the log of the drug concentration, and the half-maximal inhibitory concentration ( $IC_{50}$ ) values were interpolated from the resulting linear regression curve fit. Cell lines are ordered left to right according to increasing  $IC_{50}$  values. Error bars indicate the SE of the mean value. Mean is derived from at least three replicate experiments. Colored bars denote cell lines with a *BRAF*<sup>V600</sup> (blue) and *KRAS* (gray) short gene variations. The OC cell line ES-2, which contains a *BRAF*<sup>V600E</sup> mutation, was the most sensitive to the BRAF inhibitor vemurafenib (A). MEK inhibition with trametinib led to significant growth inhibition in the ES-2 ovarian cells but also in other cell lines harboring *KRAS*, *NF1*, and other *BRAF* mutations.



weekly nanoparticle albumin-bound paclitaxel, and then pembrolizumab in combination with epacadostat followed by topotecan plus bevacizumab (Fig 2). Upon further progression, a computed tomography (CT)-guided core biopsy was performed on an external iliac lymph node. In the absence of a therapeutic standard of care, tumor tissue was submitted to Foundation

Medicine for comprehensive genomic profiling revealing an activating *BRAF*<sup>V600E</sup> mutation as well as *ASXL1* (K580fs\*2) and *RUNX1T1* (R520H) mutations. In preclinical studies, ES-2 cells that harbored a *BRAF*<sup>V600E</sup> mutation were the most sensitive to vemurafenib (Fig 1A). Antiproliferative effects of trametinib varied significantly between individual cell lines, with

**Fig 2.** Diagram outlining the entire treatment history with corresponding CA-125 levels. CP, carboplatin and paclitaxel; GP, carboplatin and gemcitabine; PLD, liposomal doxorubicin.

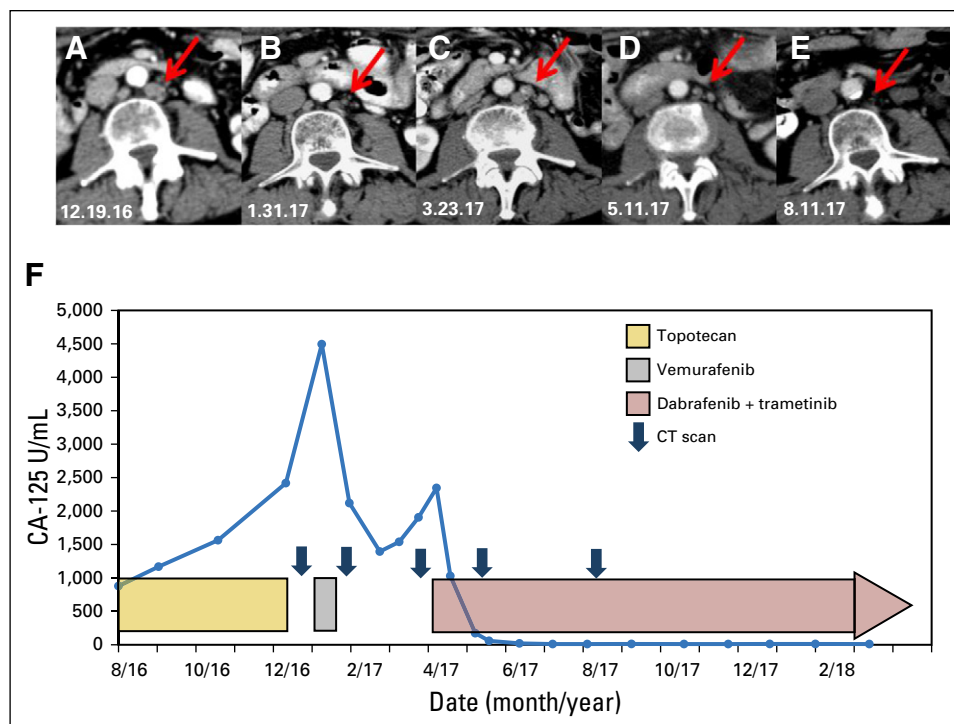


up to a 3-log-fold difference in the half-maximal inhibitory concentration values, but were most pronounced in cell lines that harbored *BRAF*, *KRAS*, or *NF1* mutations (Fig 1B). After providing informed consent, the patient was treated in accordance with the declaration of Helsinki with the BRAF inhibitor vemurafenib at the US Food and Drug Administration–approved dose of 960 mg twice a day. Because of fear of adverse effects, the patient only took half of the prescribed dose of 480 mg twice daily. Despite the dose reduction, she had to stop treatment after 13 days as a result of a profound diffuse maculopapular skin rash, which significantly improved with systemic corticosteroid medication. Despite the brevity of treatment, the patient’s CA-125 levels decreased from 4,495 to 2,119 U/mL within 21 days of initiating therapy, and a CT scan of the abdomen and pelvis revealed a partial response (PR) 29 days after commencing vemurafenib therapy.<sup>3</sup> While the patient remained off therapy awaiting insurance approval for dabrafenib plus trametinib, the CA-125 level increased again from 1,394 to 2,345 U/mL with increasing symptoms of abdominal bloating and distension as well as moderate pelvic pain. CT scans of the abdomen and pelvis done before starting new treatment showed renewed progression (Fig 3). After starting combination therapy with dabrafenib (150 mg twice a day) and trametinib (2 mg daily), the CA-125 levels decreased from 2,345 to 12 U/mL (normal range, < 35 U/mL) within a 10-week period. The patient tolerated medication well without relevant skin toxicity. A CT scan of the abdomen and pelvis showed pronounced decrease in size of the abdominal and retroperitoneal lesions consistent with a radiographic PR. In the following weeks, the patient developed mild lower extremity edema and moderate hypertension, requiring a decrease in the doses of dabrafenib from 150 mg to 75 mg twice a day and of trametinib from 2 mg to 1 mg daily. After 10 months of combination

therapy, the CA-125 levels remained in the normal range, and updated CT scans demonstrated an ongoing PR with continued reduction in size of her retroperitoneal lymphadenopathy (Fig 2). To identify additional patients who might benefit from BRAF/MEK inhibition, we analyzed a subset of 2,983 ovarian tumors from the Foundation Medicine database of more than 125,000 clinical cases that had undergone hybrid capture–based next-generation sequencing. *BRAF*<sup>V600</sup> mutations were found in 46 (1.5%) of 2,983 serous OCs, in three (4.2%) of 71 ovarian mucinous cancers, in one (3.6%) of 28 OCs with neuroendocrine differentiation, in four (5%) of 80 low-grade serous OCs, and in two (13.3%) of 15 serous borderline tumors (Table 1).

## DISCUSSION

We present a patient diagnosed with recurrent HGSOc who had received an extensive number of prior chemotherapy regimens and whose tumor was found to harbor a *BRAF*<sup>V600E</sup> mutation. Treatment with the BRAF inhibitor vemurafenib and subsequently the BRAF inhibitor dabrafenib and MEK inhibitor trametinib led to a persistent radiographic PR with normalization of CA-125 and marked symptomatic improvement. This is a remarkable response, especially in light of the patient’s extensive prior treatment and the absence of a meaningful alternative treatment option. The rapid development and widespread availability of NGS provides the opportunity to select an appropriate targeted therapy.<sup>9</sup> However, matching patients with the optimum therapy is often challenging, particularly as driver events are generally infrequent and comprehensive clinical response data are rarely available for the specific cancer type, especially with noncanonical variants. Because a conventional clinical trial design approach may not be feasible to demonstrate the effectiveness of a targeted agent in rare molecular events in a given



**Fig 3.** Computed tomography (CT) scans of the abdomen and pelvis revealed a partial response after a brief treatment with vemurafenib. For example, a left periaortic lymph node is (A) initially seen measuring 11 × 14 mm at baseline and (B) subsequently decreased to 7 × 8 mm after brief treatment with vemurafenib. (C) After holding vemurafenib, CT scans of the abdomen and pelvis showed renewed progression of the lymphadenopathy with the left periaortic node measuring 10 × 11 mm. (D) Dabrafenib and trametinib treatment again led to a significant decrease in size of the abdominal and retroperitoneal lesions with a decrease in size of the left periaortic node to 4 × 6 mm. (E) After further combination therapy, the CA-125 level remains in the normal range and updated CT scans demonstrate an ongoing partial response with continued reduction in size of the patient's left periaortic lymph node now measuring 2 × 3 mm. (F) CA-125 tumor marker levels are shown during treatment while on chemotherapy, followed by the BRAF inhibitor vemurafenib given as a single agent, followed by the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib.

cancer type, basket trials have been initiated that categorize patients' cancers on the basis of the sequencing of the tumor, rather than the organ of origin.<sup>10</sup> Nevertheless, individual case reports, such as the present description, may provide important additional evidence to support further

use of a BRAF/MEK inhibitor combination in OC found to harbor a *BRAF*<sup>V600</sup> mutation, which has been shown to be a molecular driver in a meaningful subset of other cancers, including malignant melanoma, papillary thyroid carcinomas, colorectal cancers, and non-small-cell lung cancers.<sup>11</sup> In our OC cell line panel, ES-2 cells,<sup>12</sup> which contain a *BRAF*<sup>V600E</sup> mutation, were the most sensitive to the BRAF inhibitor vemurafenib. Similarly, MEK inhibition with trametinib led to growth inhibition in ES-2 cells; however, OC cell lines with *KRAS*, *NF1*, and other *BRAF* mutations also seemed to be sensitive to MEK inhibition, suggesting potentially a broader role of MEK inhibitors in OC. However, the sensitivities of these OC cell lines differ from what is observed in melanoma cell lines, for which these inhibitors are a standard of care. For sensitive melanoma cell lines, the typical half-maximal inhibitory concentrations to vemurafenib or trametinib are less than 300 nM or in the low nanomolar range, respectively.<sup>13</sup>

**Table 1.** Frequency of *BRAF*<sup>V600E</sup> Mutations in 2,983 Ovarian Tumors

Tumor Type	Total No. of Tumors	<i>BRAF</i> <sup>V600</sup> - Positive Tumors	
		No.	%
Serous ovarian cancer	2,983	46	1.5
Ovarian mucinous cancer	71	3	4.2
Ovarian cancer with neuroendocrine differentiation	28	1	3.6
Low-grade serous ovarian cancer	80	4	5.0
Serous borderline tumors (low malignant potential)	15	2	13.3
All tumor types	3,177	56	1.7

NOTE. Profiling was previously performed on DNA extracted from formalin-fixed, paraffin-embedded samples using a hybrid capture-based next-generation sequencing platform (FoundationOne; Foundation Medicine, Cambridge, MA).<sup>8</sup>

Although it is clear that NGS-based technologies are already having an impact on patient care, for broad, widespread use to occur, several factors will need to be addressed in the coming years. Funding organizations must embrace the need for more robust predictive tumor models and new cancer research tools and assist in their development. Examples include establishing larger cancer cell line collections with integrated molecular characterization. In addition to expanding basket-type trials, we need further development of data-sharing consortia that are focused on generating an evidence base for precision cancer medicine by integrating robustly validated, broad-based genomic profiling data with clinical outcome data. This

effort will help generate the scientific evidence needed to further accelerate the adoption of precision medicine into clinical practice in oncology.<sup>14</sup> However, until further evidence from biomarker-driven clinical trials, observational databases, and improved preclinical identification of genomic determinants of response to therapy are available, case reports such as this one may help guide treatment decisions in individual patients with rare molecular genomic alterations.

DOI: <https://doi.org/10.1200/PO.18.00025>

Published online on [ascopubs.org/journal/po](http://ascopubs.org/journal/po) on June 14, 2018.

#### AUTHOR CONTRIBUTIONS

**Conception and design:** Maira P. Campos, Melissa Cohen, Dennis J. Slamon, Julia Elvin, Gottfried E. Konecny

**Provision of study materials or patients:** Maira P. Campos, Melissa Cohen, Erika Von Euw, Gottfried E. Konecny

**Collection and assembly of data:** Maira P. Campos, Melissa Cohen, Erika Von Euw, Victor Velculescu, Jennifer L. Kujak, Julia Elvin, Gottfried E. Konecny

**Data analysis and interpretation:** Maira P. Campos, Erika Von Euw, Victor Velculescu, Dylan Conklin, Dorothy Hallberg, Julia Elvin, Gottfried E. Konecny

**Manuscript writing:** All authors

**Final approval of manuscript:** All authors

**Accountable for all aspects of the work:** All authors

**Victor Velculescu**

**Leadership:** Personal Genome Diagnostics

**Stock and Other Ownership Interests:** Personal Genome Diagnostics, Ignyta

**Consulting or Advisory Role:** Quintiles, Janssen Diagnostics, Ignyta, Personal Genome Diagnostics

**Patents, Royalties, Other Intellectual Property:** Receive royalties for discoveries our group has made at Johns Hopkins that have been licensed by Qiagen; receive royalties for discoveries our group has made at Johns Hopkins that have been licensed by Myriad; receive royalties for discoveries our group has made at Johns Hopkins that have been licensed by Personal Genome Diagnostics; receive royalties for discoveries our group has made at Johns Hopkins that have been licensed by Genzyme; receive royalties for discoveries our group has made at Johns Hopkins that have been licensed by Roche

**Jennifer L. Kujak**

**Honoraria:** Eli Lilly, Blue Earth Diagnostics

**Consulting or Advisory Role:** Eli Lilly, Blue Earth Diagnostics

**Speakers' Bureau:** Eli Lilly, Blue Earth Diagnostics

**Dylan Conklin**

No relationship to disclose

**Dorothy Hallberg**

No relationship to disclose

**Dennis J. Slamon**

**Leadership:** Biomarin

**Stock and Other Ownership Interests:** Pfizer

**Honoraria:** Novartis

**Consulting or Advisory Role:** Novartis, Eli Lilly

**Research Funding:** Novartis, Pfizer

**Travel, Accommodations, Expenses:** Pfizer, Biomarin, Novartis

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to [www.asco.org/rwc](http://www.asco.org/rwc) or [ascopubs.org/po/author=center](http://ascopubs.org/po/author=center).

**Maira P. Campos**

No relationship to disclose

**Melissa Cohen**

No relationship to disclose

**Erika Von Euw**

No relationship to disclose



**Julia Elvin**  
**Employment:** Foundation Medicine  
**Stock and Other Ownership Interests:** Foundation Medicine

**Gottfried E. Konecny**  
**Stock and Other Ownership Interests:** Foundation Medicine  
**Speakers' Bureau:** Clovis Oncology, AstraZeneca, Tesaro  
**Research Funding:** Merck  
**Travel, Accommodations, Expenses:** Novartis

### Affiliations

**Maira P. Campos, Melissa Cohen, Dennis J. Slamon, and Gottfried E. Konecny**, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles; **Maira P. Campos, Erika Von Euw, Dylan Conklin, Dennis J. Slamon, and Gottfried E. Konecny**, University of California, Los Angeles Translational Oncology Research Laboratory, University of California, Los Angeles, Santa Monica; **Jennifer L. Kujak**, Rolling Oaks Radiology, Thousand Oaks, CA; **Victor Velculescu and Dorothy Hallberg**, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD; and **Julia Elvin**, Foundation Medicine, Cambridge, MA.

### Support

Supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

### REFERENCES

1. Jemal A, Siegel R, Xu J, et al: Cancer statistics, 2010. *CA Cancer J Clin* 60:277-300, 2010
2. Cancer Genome Atlas Research Network: Integrated genomic analyses of ovarian carcinoma. *Nature* 474:609-615, 2011
3. Bamford S, Dawson E, Forbes S, et al: The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. *Br J Cancer* 91:355-358, 2004
4. Solit DB, Garraway LA, Pratils CA, et al: BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 439:358-362, 2006
5. Chapman PB, Hauschild A, Robert C, et al: Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 364:2507-2516, 2011
6. Larkin J, Ascierto PA, Dréno B, et al: Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N Engl J Med* 371:1867-1876, 2014
7. Ihnen M, zu Eulenburg C, Kolarova T, et al: Therapeutic potential of the poly(ADP-ribose) polymerase inhibitor rucaparib for the treatment of sporadic human ovarian cancer. *Mol Cancer Ther* 12:1002-1015, 2013
8. Frampton GM, Fichtenholtz A, Otto GA, et al: Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 31:1023-1031, 2013
9. Ross JS, Ali SM, Wang K, et al: Comprehensive genomic profiling of epithelial ovarian cancer by next generation sequencing-based diagnostic assay reveals new routes to targeted therapies. *Gynecol Oncol* 130:554-559, 2013
10. Barroilhet L, Matulonis U: The NCI-MATCH trial and precision medicine in gynecologic cancers. *Gynecol Oncol* 148:585-590, 2018
11. Cantwell-Dorris ER, O'Leary JJ, Sheils OM: BRAFV600E: Implications for carcinogenesis and molecular therapy. *Mol Cancer Ther* 10:385-394, 2011
12. Domcke S, Sinha R, Levine DA, et al: Evaluating cell lines as tumour models by comparison of genomic profiles. *Nat Commun* 4:2126, 2013
13. Stones CJ, Kim JE, Joseph WR, et al: Comparison of responses of human melanoma cell lines to MEK and BRAF inhibitors. *Front Genet* 4:66, 2013
14. AACR Project GENIE Consortium: AACR Project GENIE: Powering precision medicine through an international consortium. *Cancer Discov* 7:818-831, 2017