



ORIGINAL ARTICLE

CCNE1 and survival of patients with tubo-ovarian high-grade serous carcinoma: An Ovarian Tumor Tissue Analysis consortium study

Eun-Young Kang MD¹ | Ashley Weir BAdvSc (Hons)^{2,3,4} | Nicola S. Meagher MPH^{2,5}  | Kyo Farrington MD¹ | Gregg S. Nelson MD, PhD⁶ | Prafull Ghatage MD⁶ | Cheng-Han Lee MD, PhD⁷  | Marjorie J. Riggan BSc⁸ | Adelyn Bolithon BSc (Hons)^{3,9} | Gordana Popovic PhD¹⁰ | Betty Leung MBBS, FRCPA¹¹ | Katrina Tang MBBS, FRCPA¹² | Neil Lambie MB ChB, Dip Obs, FRCPA¹³ | Joshua Millstein PhD¹⁴ | Jennifer Alsop BA¹⁵ | Michael S. Anglesio PhD^{16,17} | Beyhan Ataseven MD, PhD^{18,19} | Ellen Barlow PhD²⁰ | Matthias W. Beckmann MD²¹ | Jessica Berger MD²² | Christiani Bisinotto MD²³ | Hans Bösmüller MD, PhD²⁴ | Jessica Boros BSc (Hons)^{25,26,27} | Alison H. Brand MD^{26,27} | Angela Brooks-Wilson PhD²⁸ | Sara Y. Brucker MD²⁹ | Michael E. Carney MD³⁰ | Yovanni Casablanca MD³¹ | Alicia Cazorla-Jiménez MD³² | Paul A. Cohen MD^{33,34} | Thomas P. Conrads PhD³⁵ | Linda S. Cook PhD^{36,37} | Penny Coulson BA (Hons)³⁸ | Madeleine Courtney-Brooks MD, MPH²² | Daniel W. Cramer MD, ScD^{39,40} | Philip Crowe MB BS, DPhil, FRACS^{11,41} | Julie M. Cunningham PhD⁴² | Cezary Cybulski MD, PhD⁴³ | Kathleen M. Darcy PhD^{44,45} | Mona A. El-Bahrawy PhD⁴⁶ | Esther Elishaev MD⁴⁷ | Ramona Erber MD⁴⁸ | Rhonda Farrell MBBS⁴⁹ | Sian Fereday BSc, BA^{50,51}  | Anna Fischer MD²⁴ | María J. García PhD⁵² | Simon A. Gayther PhD⁵³ | Aleksandra Gentry-Maharaj PhD⁵⁴ | C. Blake Gilks MD⁵⁵ | AOCS Group^{25,50,56} | Marcel Grube MD²⁹ | Paul R. Harnett MB, BS, PhD^{27,57} | Shariska Petersen Harrington MD⁵⁸ | Philipp Harter MD^{18,59} | Arndt Hartmann MD, PhD⁴⁸ | Jonathan L. Hecht MD, PhD⁶⁰ | Sebastian Heikau MD, PhD⁶¹ | Alexander Hein MD²¹ | Florian Heitz MD^{18,59,61} | Joy Hendley⁵⁰ | Brenda Y. Hernandez PhD, MPH⁶² | Susanna Hernando Polo MD⁶³ |

Eun-Young Kang and Ashley Weir contributed equally to the study.

Susan J. Ramus and Martin Köbel contributed equally to the study.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. Cancer published by Wiley Periodicals LLC on behalf of American Cancer Society.

Sabine Heublein MD, PhD⁶⁴ | Akira Hirasawa MD, PhD⁶⁵ |
 Estrid Høgdall PhD, DMSc⁶⁶ | Claus K. Høgdall MD, DSc⁶⁷ | Hugo M. Horlings MD⁶⁸ |
 David G. Huntsman MD^{16,69} | Tomasz Huzarski PhD^{43,70} | Andrea Jewell MD⁵⁸ |
 Mercedes Jimenez-Linan MD⁷¹ | Michael E. Jones PhD³⁸ |
 Scott H. Kaufmann MD, PhD⁷² | Catherine J. Kennedy BSc (Hons)^{25,26,27}  |
 Dineo Khabele MD⁷³ | Felix K. F. Kommos MD⁷⁴ |
 Roy F. P. M. Kruitwagen MD, PhD^{75,76} | Diether Lambrechts PhD^{77,78} |
 Nhu D. Le PhD⁷⁹ | Marcin Lener MD⁸⁰ | Jenny Lester MPH⁸¹ |
 Yee Leung MD^{82,83,84} | Anna Linder PhD⁸⁵ | Liselore Loverix MD⁸⁶ |
 Jan Lubiński MD, PhD⁴³ | Rashna Madan MBBS⁸⁷ | G. Larry Maxwell MD⁸⁸ |
 Francesmary Modugno PhD, MPH^{22,89,90} | Susan L. Neuhausen PhD⁹¹ |
 Alexander Olawaiye MD²² | Siel Olbrecht MD⁸⁶ | Sandra Orsulic PhD⁸¹ |
 José Palacios MD, PhD⁹² | Celeste Leigh Pearce PhD⁹³ | Malcolm C. Pike PhD^{94,95} |
 Carmel M. Quinn DPhil, MPH⁹⁶ |
 Ganendra Raj Mohan MBBS, FRCOG, FRANZCOG, CGO^{33,83} |
 Cristina Rodríguez-Antona PhD^{97,98} | Matthias Ruebner PhD²¹ |
 Andy Ryan PhD^{54,99} | Stuart G. Salfinger MD³³ | Naoko Sasamoto MD, MPH³⁹  |
 Joellen M. Schildkraut PhD¹⁰⁰  | Minouk J. Schoemaker PhD³⁸ | Mitul Shah MSc¹⁵ |
 Raghwa Sharma MBBS, FRCPA¹⁰¹ | Yurii B. Shvetsov PhD⁶² | Naveena Singh MD¹⁰² |
 Gabe S. Sonke MD, PhD¹⁰³ | Linda Steele BS⁹¹ | Colin J. R. Stewart MD¹⁰⁴ |
 Karin Sundfeldt MD, PhD⁸⁵ | Anthony J. Swerdlow DSc^{38,105} |
 Aline Talhouk PhD^{16,17}  | Adeline Tan MBBS (Hons), FRCPA^{82,106} |
 Sarah E. Taylor MD²² | Kathryn L. Terry ScD^{39,40} | Aleksandra Tołoczko MD¹⁰⁷ |
 Nadia Traficante BSc^{50,51} | Koen K. Van de Vijver MD, PhD^{108,109} |
 Maaïke A. van der Aa MD, PhD¹¹⁰ | Toon Van Gorp MD, PhD⁸⁶ |
 Els Van Nieuwenhuysen PhD⁸⁶ | Lilian van-Wagensveld PhD^{75,76,110} |
 Ignace Vergote MD⁸⁶  | Robert A. Vierkant MS¹¹¹ | Chen Wang PhD¹¹² |
 Lynne R. Wilkens DrPH, MS⁶² | Stacey J. Winham PhD¹¹² | Anna H. Wu PhD⁹⁵ |
 Javier Benitez PhD^{98,113} | Andrew Berchuck MD⁸ |
 Francisco J. Candido dos Reis MD, PhD²³ | Anna DeFazio PhD^{5,25,26,27} |
 Peter A. Fasching MD²¹ | Ellen L. Goode PhD, MPH¹¹⁴ | Marc T. Goodman PhD¹¹⁵ |
 Jacek Gronwald MD, PhD⁴³ | Beth Y. Karlan MD⁸¹ | Stefan Kommos MD²⁹ |
 Usha Menon MD⁵⁴ | Hans-Peter Sinn MD, PhD⁷⁴ | Annette Staebler MD²⁴ |
 James D. Brenton MD¹¹⁶ | David D. Bowtell PhD^{50,51} | Paul D. P. Pharoah PhD^{15,117} |
 Susan J. Ramus PhD^{2,3} | Martin Köbel MD¹ 

¹Department of Pathology and Laboratory Medicine, University of Calgary, Foothills Medical Center, Calgary, Alberta, Canada

²School of Clinical Medicine, UNSW Medicine and Health, University of NSW Sydney, Sydney, New South Wales, Australia

³Adult Cancer Program, Lowy Cancer Research Centre, University of NSW Sydney, Sydney, New South Wales, Australia

⁴The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

- ⁵The Daffodil Centre, The University of Sydney, A Joint Venture With Cancer Council NSW, Sydney, New South Wales, Australia
- ⁶Department of Oncology, Division of Gynecologic Oncology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
- ⁷Department of Pathology and Laboratory Medicine, University of Alberta, Edmonton, Alberta, Canada
- ⁸Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Duke University Medical Center, Durham, North Carolina, USA
- ⁹School of Women's and Children's Health, Faculty of Medicine and Health, University of NSW Sydney, Sydney, New South Wales, Australia
- ¹⁰Stats Central, Mark Wainwright Analytical Centre, University of NSW Sydney, Sydney, New South Wales, Australia
- ¹¹Prince of Wales Clinical School, UNSW Medicine and Health, University of NSW Sydney, Sydney, New South Wales, Australia
- ¹²Department of Anatomical Pathology, Prince of Wales Hospital, Sydney, New South Wales, Australia
- ¹³Canterbury Health Laboratories, Christchurch, New Zealand
- ¹⁴Division of Biostatistics, Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, California, USA
- ¹⁵Department of Oncology, Centre for Cancer Genetic Epidemiology, University of Cambridge, Cambridge, UK
- ¹⁶Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, British Columbia, Canada
- ¹⁷British Columbia's Gynecological Cancer Research Team (OVCARE), University of British Columbia, BC Cancer, and Vancouver General Hospital, Vancouver, British Columbia, Canada
- ¹⁸Department of Gynecology and Gynecologic Oncology, Evangelische Kliniken Essen-Mitte (KEM), Essen, Germany
- ¹⁹Department of Obstetrics and Gynecology, Ludwig Maximilian University Munich, Munich, Germany
- ²⁰Gynaecological Cancer Centre, Royal Hospital for Women, Sydney, New South Wales, Australia
- ²¹Department of Gynecology and Obstetrics, Comprehensive Cancer Center Erlangen-EMN, Friedrich-Alexander University Erlangen-Nuremberg, University Hospital Erlangen, Erlangen, Germany
- ²²Division of Gynecologic Oncology, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
- ²³Department of Gynecology and Obstetrics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil
- ²⁴Institute of Pathology and Neuropathology, Tuebingen University Hospital, Tuebingen, Germany
- ²⁵Centre for Cancer Research, The Westmead Institute for Medical Research, University of Sydney, Sydney, New South Wales, Australia
- ²⁶Department of Gynaecological Oncology, Westmead Hospital, Sydney, New South Wales, Australia
- ²⁷Discipline of Obstetrics and Gynaecology, The University of Sydney, Sydney, New South Wales, Australia
- ²⁸Canada's Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, British Columbia, Canada
- ²⁹Department of Women's Health, Tuebingen University Hospital, Tuebingen, Germany
- ³⁰Department of Obstetrics and Gynecology, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA
- ³¹Uniformed Services of the Health Sciences Gynecologic Cancer Center of Excellence, Bethesda, Maryland, USA
- ³²Pathology Department, Fundación Jiménez Díaz, Madrid, Spain
- ³³Department of Gynaecological Oncology, St John of God Subiaco Hospital, Subiaco, Western Australia, Australia
- ³⁴Division of Obstetrics and Gynaecology, Medical School, University of Western Australia, Crawley, Western Australia, Australia
- ³⁵Women's Health Integrated Research Center, Inova Health System, Falls Church, Virginia, USA
- ³⁶Epidemiology, School of Public Health, University of Colorado, Aurora, Colorado, USA
- ³⁷Community Health Sciences, University of Calgary, Calgary, Alberta, Canada
- ³⁸Division of Genetics and Epidemiology, The Institute of Cancer Research, London, UK
- ³⁹Obstetrics and Gynecology Epidemiology Center, Department of Obstetrics and Gynecology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA
- ⁴⁰Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
- ⁴¹Department of Surgery, Prince of Wales Private Hospital, Randwick, New South Wales, Australia
- ⁴²Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA
- ⁴³Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland
- ⁴⁴Gynecologic Cancer Center of Excellence, Department of Gynecologic Surgery and Obstetrics, Uniformed Services University of the Health Sciences, Walter Reed National Military Medical Center, Bethesda, Maryland, USA
- ⁴⁵Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc, Bethesda, Maryland, USA
- ⁴⁶Department of Metabolism, Digestion and Reproduction, Imperial College London, Hammersmith Hospital, London, UK
- ⁴⁷Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
- ⁴⁸Institute of Pathology, Comprehensive Cancer Center Erlangen-EMN, Friedrich-Alexander University Erlangen-Nuremberg, University Hospital Erlangen, Erlangen, Germany

- ⁴⁹Prince of Wales Private Hospital, Randwick, New South Wales, Australia
- ⁵⁰Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia
- ⁵¹Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria, Australia
- ⁵²Computational Oncology Group, Structural Biology Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- ⁵³Center for Bioinformatics and Functional Genomics and the Cedars Sinai Genomics Core, Cedars-Sinai Medical Center, Los Angeles, California, USA
- ⁵⁴MRC Clinical Trials Unit, Institute of Clinical Trials & Methodology, University College London, London, UK
- ⁵⁵Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada
- ⁵⁶QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia
- ⁵⁷Crown Princess Mary Cancer Centre, Westmead Hospital, Sydney, New South Wales, Australia
- ⁵⁸Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, The University of Kansas Medical Center, Kansas City, Kansas, USA
- ⁵⁹Department of Gynecology and Gynecological Oncology, HSK, Dr. Horst-Schmidt Klinik, Wiesbaden, Wiesbaden, Germany
- ⁶⁰Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA
- ⁶¹Center for Pathology, Evangelische Kliniken Essen-Mitte, Essen, Germany
- ⁶²University of Hawaii Cancer Center, Honolulu, Hawaii, USA
- ⁶³Medical Oncology Service, Hospital Universitario Funcación Alcorcón, Alcorcón, Spain
- ⁶⁴Department of Obstetrics and Gynecology, University Hospital Heidelberg, Heidelberg, Germany
- ⁶⁵Department of Clinical Genomic Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
- ⁶⁶Department of Pathology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark
- ⁶⁷Department of Gynaecology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
- ⁶⁸Division of Molecular Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
- ⁶⁹Department of Molecular Oncology, BC Cancer Research Centre, Vancouver, British Columbia, Canada
- ⁷⁰Department of Genetics and Pathology, University of Zielona Gora, Zielona Gora, Poland
- ⁷¹Department of Histopathology, Addenbrooke's Hospital, Cambridge, UK
- ⁷²Division of Oncology Research and Department of Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, Rochester, Minnesota, USA
- ⁷³Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Washington University in St. Louis, St. Louis, Missouri, USA
- ⁷⁴Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany
- ⁷⁵Department of Obstetrics and Gynecology, Maastricht University Medical Centre, Maastricht, The Netherlands
- ⁷⁶GROW – School for Oncology and Reproduction, Maastricht University Medical Center, Maastricht, The Netherlands
- ⁷⁷Department of Human Genetics, Laboratory for Translational Genetics, KU Leuven, Leuven, Belgium
- ⁷⁸VIB Center for Cancer Biology, VIB, Leuven, Belgium
- ⁷⁹Cancer Control Research, BC Cancer Agency, Vancouver, British Columbia, Canada
- ⁸⁰International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University in Szczecin, Szczecin, Poland
- ⁸¹David Geffen School of Medicine, Department of Obstetrics and Gynecology, University of California at Los Angeles, Los Angeles, California, USA
- ⁸²Division of Obstetrics and Gynaecology, Faculty of Health and Medical Sciences, University of Western Australia, Crawley, Western Australia, Australia
- ⁸³Department of Gynaecological Oncology, King Edward Memorial Hospital, Subiaco, Western Australia, Australia
- ⁸⁴Australia New Zealand Gynaecological Oncology Group, Camperdown, Australia
- ⁸⁵Department of Obstetrics and Gynecology, Inst of Clinical Science, Sahlgrenska Center for Cancer Research, University of Gothenburg, Gothenburg, Sweden
- ⁸⁶Division of Gynecologic Oncology, Department of Gynecology and Obstetrics, Leuven Cancer Institute, Leuven, Belgium
- ⁸⁷Department of Pathology and Laboratory Medicine, The University of Kansas Medical Center, Kansas City, Kansas, USA
- ⁸⁸Inova Health System Women's Service Line, Falls Church, Virginia, USA
- ⁸⁹Department of Epidemiology, University of Pittsburgh School of Public Health, Pittsburgh, Pennsylvania, USA
- ⁹⁰Women's Cancer Research Center, Magee-Womens Research Institute and Hillman Cancer Center, Pittsburgh, Pennsylvania, USA
- ⁹¹Department of Population Sciences, Beckman Research Institute of City of Hope, Duarte, California, USA
- ⁹²Department of Pathology, Hospital Ramón y Cajal, Instituto Ramon y Cajal de Investigación Sanitaria (IRYCIS), CIBERONC, Universidad de Alcalá, Madrid, Spain
- ⁹³Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan, USA
- ⁹⁴Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, New York, USA
- ⁹⁵Department of Population Health and Public Health Sciences, Keck School of Medicine, University of Southern California Norris Comprehensive Cancer Center, Los Angeles, California, USA
- ⁹⁶The Health Precincts Biobank, UNSW Biospecimen Services, Mark Wainwright Analytical Centre, University of NSW Sydney, Sydney, New South Wales, Australia

- ⁹⁷Hereditary Endocrine Cancer Group, Spanish National Cancer Research Center (CNIO), Madrid, Spain
- ⁹⁸Centre for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain
- ⁹⁹Women's Cancer, Institute for Women's Health, University College London, London, UK
- ¹⁰⁰Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA
- ¹⁰¹Tissue Pathology and Diagnostic Oncology, Westmead Hospital, Sydney, New South Wales, Australia
- ¹⁰²Department of Pathology, Barts Health National Health Service Trust, London, UK
- ¹⁰³Department of Medical Oncology, The Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands
- ¹⁰⁴School for Women's and Infants' Health, University of Western Australia, Perth, Australia
- ¹⁰⁵Division of Breast Cancer Research, The Institute of Cancer Research, London, UK
- ¹⁰⁶Gynaepath WA, Clinipath (Sonic Healthcare), Osbourne Park, Australia
- ¹⁰⁷Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland
- ¹⁰⁸Department of Pathology, Ghent University Hospital, Cancer Research Institute Ghent (CRIG), Ghent, Belgium
- ¹⁰⁹Department of Pathology, Antwerp University Hospital, Antwerp, Belgium
- ¹¹⁰Department of Research, Netherlands Comprehensive Cancer Organization (IKNL), Utrecht, The Netherlands
- ¹¹¹Department of Quantitative Health Sciences, Division of Clinical Trials and Biostatistics, Mayo Clinic, Rochester, Minnesota, USA
- ¹¹²Department of Quantitative Health Sciences, Division of Computational Biology, Mayo Clinic, Rochester, Minnesota, USA
- ¹¹³Human Genetics Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain
- ¹¹⁴Department of Quantitative Health Sciences, Division of Epidemiology, Mayo Clinic, Rochester, Minnesota, USA
- ¹¹⁵Cancer Prevention and Control Program, Cedars-Sinai Cancer, Cedars-Sinai Medical Center, Los Angeles, California, USA
- ¹¹⁶Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, UK
- ¹¹⁷Department of Public Health and Primary Care, Centre for Cancer Genetic Epidemiology, University of Cambridge, Cambridge, UK

Correspondence

Susan J. Ramus, Discipline of Women's Health, Adult Cancer Program, Lowy Building Room 219, Level 2, School of Medicine, UNSW Medicine & Health, Sydney, NSW 2052, Australia.
Email: s.ramus@unsw.edu.au

Martin Köbel, Department of Pathology and Laboratory Medicine, University of Calgary, 1403 29 ST NW, Calgary, AB T2N 2T9, Canada.
Email: mkoebel@ucalgary.ca

Funding information

National Cancer Institute, Grant/Award Number: R01CA172404; Alberta Precision Laboratories, Grant/Award Number: RS17-601, RS10-526
Open access publishing facilitated by University of New South Wales, as part of the Wiley - University of New South Wales agreement via the Council of Australian University Librarians.

Abstract

Background: Cyclin E1 (CCNE1) is a potential predictive marker and therapeutic target in tubo-ovarian high-grade serous carcinoma (HGSC). Smaller studies have revealed unfavorable associations for CCNE1 amplification and CCNE1 overexpression with survival, but to date no large-scale, histotype-specific validation has been performed. The hypothesis was that high-level amplification of CCNE1 and CCNE1 overexpression, as well as a combination of the two, are linked to shorter overall survival in HGSC.

Methods: Within the Ovarian Tumor Tissue Analysis consortium, amplification status and protein level in 3029 HGSC cases and mRNA expression in 2419 samples were investigated.

Results: High-level amplification (>8 copies by chromogenic *in situ* hybridization) was found in 8.6% of HGSC and overexpression (>60% with at least 5% demonstrating strong intensity by immunohistochemistry) was found in 22.4%. CCNE1 high-level amplification and overexpression both were linked to shorter overall survival in multivariate survival analysis adjusted for age and stage, with hazard stratification by study (hazard ratio [HR], 1.26; 95% CI, 1.08-1.47, $p = .034$, and HR, 1.18; 95% CI, 1.05-1.32, $p = .015$, respectively). This was also true for cases with combined high-level amplification/overexpression (HR, 1.26; 95% CI, 1.09-1.47, $p = .033$). CCNE1 mRNA expression was not associated with overall survival (HR, 1.00 per 1-SD increase; 95% CI, 0.94-1.06; $p = .58$). CCNE1 high-level amplification is mutually exclusive with the presence of germline BRCA1/2 pathogenic variants and shows an inverse association to RB1 loss.

Conclusion: This study provides large-scale validation that *CCNE1* high-level amplification is associated with shorter survival, supporting its utility as a prognostic biomarker in HGSC.

KEYWORDS

CCNE1 amplification, cyclin E1 expression, high-grade serous carcinoma, ovarian cancer, prognosis

INTRODUCTION

Cyclin E1 (*CCNE1*) is a potential predictive marker and therapeutic target in tubo-ovarian high-grade serous carcinoma (HGSC).^{1,2} *CCNE1* has three main functions in cell-cycle progression.³ First, it is involved in the formation of prereplication minichromosome maintenance protein complexes, which bind origins of DNA replications as cells reenter G1- from G0-phase of the cell cycle. Second, by forming a complex, it activates the cyclin-dependent kinase CDK2 to phosphorylate several targets including RB1, which subsequently abandons its inhibition of E2F transcription factors and initiates the transition from G1 to S phase.³ CDK2 inhibition by cyclin-dependent kinase inhibitors 1 (CDKN1a/p21) is dependent on normal TP53 function. Third, the CDK2/*CCNE1* complex promotes centrosome duplication.^{3,4} Normal *CCNE1* protein levels are tightly regulated, peaking in late G1 and decreasing as cells progress through S phase.⁵ In neoplasia, *CCNE1* protein overexpression is uncoupled from the cell cycle.⁶ Constitutive overexpression of *CCNE1*, but not of *CCND1* or *CCNA*, induces chromosomal instability and a modest degree of polyploidy.⁶ The mechanisms by which *CCNE1* causes chromosomal instability are not entirely understood, but it has been suggested that cells with deregulated *CCNE1* prematurely enter S phase with inadequate nucleotide pools, causing replication stress with faulty replication forks engendering DNA double-stranded breaks.^{7,8}

In ovarian carcinoma, *CCNE1* amplification has been associated with resistance to platinum-based chemotherapy and shorter overall survival.^{9,10} However, the cutoff for amplification varies among studies. Larger studies such as The Cancer Genome Atlas project reported only a suggestive trend toward shorter overall survival ($p = .0718$) and another study of 179 HGSC showed evidence for a significant association only with progression-free survival.^{11,12} Amplification of the chromosomal region 19q12 containing the *CCNE1* gene is common (20%) in HGSC, which across all tumor sites ranks third in frequency after endometrial carcinosarcoma and urothelial carcinoma.¹³ *CCNE1* amplification is inversely associated with germline pathogenic *BRCA1/2* variants, which becomes mutually exclusive for high-level amplifications (defined by >8 copies).^{14,15} *CCNE1* high-level amplified HGSC require proficient homologous recombination, including *BRCA1/2* function to maintain cell viability.^{14,15} *CCNE1* high-level amplification is the lead alteration for both the copy number signature 6 and the fold-back inversion mutation signature, which characterize homologous recombination-proficient HGSC.^{16,17} Patients with HGSC and homologous recombination-proficient tumors

do not respond well to chemotherapy or poly (ADP-ribose) polymerase (PARP) inhibitors. For example, PARP maintenance therapy for patients with homologous recombination-proficient HGSC and partial chemotherapy response resulted in a median progression-free survival of 8.3 months compared with 21.9 months for patients with homologous recombination-deficient HGSC.¹⁸

Although no association of *CCNE1* mRNA expression with survival in HGSC has been observed,^{15,19} *CCNE1* protein overexpression has been associated with unfavorable outcomes in ovarian carcinomas, albeit only in studies conducted before the era of histotype-specific analysis.^{20–22} Two recent studies suggested that the combination of *CCNE1* amplifications and *CCNE1* overexpression is associated with shorter survival.^{15,23} We recently validated a *CCNE1* chromogenic *in situ* hybridization (CISH) assay orthogonally against other copy number assays to be applicable on tissue microarrays and refined the cutoff for immunohistochemistry to detect *CCNE1* high-level gene amplifications.¹⁵

Here, our objectives were to validate previously reported associations of *CCNE1* alterations with overall survival; assess correlations between *CCNE1* high-level gene amplifications, *CCNE1* mRNA, and *CCNE1* protein expression; and explore associations with selected biomarkers in a large cohort of HGSC samples from the international Ovarian Tumor Tissue Analysis (OTTA) consortium.

METHODS

Study cohort

Twenty studies from the OTTA consortium participated in the current study.²⁴ Each study enrolling patients received local ethics review board approval (Table S1). Tissue microarrays were constructed from formalin-fixed paraffin-embedded tumor specimens obtained from debulking surgery representing each tumor with one to three cores, 0.6 to 1.0 mm in size. For both CISH and immunohistochemistry (IHC), data were successfully obtained in 3029 samples from patients with HGSC. Clinical covariates, time to follow up, and status were centrally standardized. Cases were collected during the pre-PARP inhibitor era. Platinum-based chemotherapy was given in the majority as adjuvant therapy after primary debulking surgery or as neoadjuvant chemotherapy. Information on specific drugs was not collected. Previously generated IHC data within the OTTA consortium for TP53, CDKN2A, and RB1 were used.^{25–27}

CCNE1 DNA CISH

A previously published in-house CISH protocol using a commercial digoxigenin (DIG)-labeled CCNE1 DNA probe (Empire Genomics, Buffalo, NY, USA) was used.¹⁵ Deparaffinized 4- μ m tissue microarray sections were pretreated with proteinase K (3 min) and citrate-based antigen retrieval buffer at 80°C (1 h) followed by pepsin (45 sec), and then dehydrated and air-dried. Hybridization with the DIG-labeled CCNE1 probe was performed at 37°C for 16 to 18 hours in HybEZ II (Advanced Cell Diagnostics, Minneapolis, MN, USA). A levamisole solution was used (15 min) to remove endogenous alkaline phosphatase activity, followed by a blocking solution (30 min) of 10% normal sheep serum, 2% bovine serum albumin, and 0.05% Tween-20. An alkaline phosphatase-conjugated sheep anti-DIG antibody (dilution 1:800; Roche, Basel, Switzerland) was incubated for 2 h. An alkaline phosphatase substrate was applied, and the reaction was stopped with 50 mM Tris, 150 mM NaCl, and 10 mM KCl buffer when slides reached the desired intensity of staining. Counterstaining was performed with hematoxylin.

CCNE1 immunohistochemistry

Four- μ m sections from tissue microarrays were deparaffinized, rehydrated, and subjected to heat-induced epitope retrieval on the DAKO Omnis platform (Agilent Technologies, Santa Clara, CA, USA), followed by incubation with the CCNE1 antibody (1:600, clone EP126, Cell Marque, Rocklin, CA, USA; 30-10R-30) at room temperature and in the EnVision FLEX (Agilent Technologies). The reaction was visualized using 3,3-diaminobenzidine tetrahydrochloride for 10 min and counterstained with hematoxylin.

CCNE1 CISH and IHC scoring

The CCNE1 CISH assay was previously orthogonally validated to detect CCNE1 high-level amplifications (presence of clusters >8 copies) against a digital polymerase chain reaction and nCounter Cancer CN Assay.¹⁵ CCNE1 protein expression showed a wide and relatively even distribution from 5% to 90% of positive tumor cells, but previous receiver operating characteristic curve analysis established an optimal cutoff for IHC to detect high-level amplification at >60% overall staining cells with at least 5% showing strong intensity.¹⁵ The interpretation of tissue microarrays was performed by three pathologists. Training was provided on a set of 90 HGSC cases guided by illustrated examples. Subsequently, interobserver reproducibility was tested on 415 cases. The interobserver observer reproducibility for paired observers achieved a Cohen's kappa of 0.48, 0.55, and 0.77 for CISH and 0.65, 0.75, and 0.85 for IHC using a binary categorization. Subsequently, examples of discordant cases were discussed at a multiheaded microscope to further align

interpretational thresholds and equivocal categories were allowed both for CISH and IHC.¹⁵ Each observer subsequently scored approximately one-third of the cases by using the following criteria for CISH: score 0, no clusters = negative for high-level amplification (CCNE1^{nonamp}); score 1, equivocal favor negative; score 2, equivocal favor high; and score 3, nuclear clusters of CISH signal = high-level amplification (CCNE1^{amp}); and for IHC score 0, <60% positive tumor cells (CCNE1^{lo}); score 1, equivocal favor low; score 2, equivocal favor high; and score 3, \geq 60% positive tumor cells with at least 5% strongly staining cells (CCNE1^{hi}).

CCNE1 mRNA expression by NanoString

Formalin-fixed paraffin-embedded tumor specimens ($n = 2419$) with partial overlap to the previous specimens (1612/3029) were obtained from additional cores or sections.²⁸ RNA extraction methods, assay run parameters, data processing, and control/reference samples were as previously described.²⁹ CCNE1 mRNA expression was assessed using the NanoString nCounter technology; the CCNE1 target sequence was CCTCCAGACACCAGTGCCTGCCGATGCTGCT ATGGAAGGTGCTACTTGACCTAAGGGACTCCACAACAACAAA GCTTGAAGCTGTGGAGGGCCAC, and CCNE1 mRNA data were normalized against housekeeping genes.²⁹ Quality assurance of the assay was previously performed with high duplicate sample correlation.^{19,30}

Statistical analyses

Correlations between CCNE1 mRNA, gene amplification (ISH), and protein (IHC) overexpression were measured using Pearson correlation coefficients. Chi-square proportions testing was undertaken to evaluate clinical and molecular variables across CCNE1 combinations. Univariate and multivariate survival analyses of CCNE1 profiles were performed. Overall survival (death from any cause) was the primary end point. Potential survival bias introduced by the time between diagnosis and study enrollment was moderated by left truncation. Deaths potentially unrelated to HGSC were right censored at 10 years from diagnosis. The Kaplan-Meier method, alongside log-rank testing, was used to assess overall survival by CCNE1 profile. Multivariate Cox proportional hazards regression modeling, stratified by the OTTA study, complemented this analysis through estimation of hazard ratios (HRs) with 95% CIs. The covariates, age, stage, completeness of surgical cytoreduction (residual disease vs. no residual disease [sensitivity analysis]), and CCNE1 profiles were adjusted for, and different baseline hazards of OTTA studies were stratified, in multivariate models. Scaled Schoenfeld residuals assessed the assumption of proportional hazards. All statistical analyses were performed using RStudio v1.1.463 or GraphPad Prism v7.02. Statistical significance was defined by $p < .05$.

RESULTS

Prevalence of CCNE1 high-level amplification and association with overall survival

CCNE1 CISH showed high-level amplification (score 3) in 259/3029 (8.6%) cases and 2426/3029 (80.2%) demonstrated no evidence of amplification (score 0). The remainder were equivocal with 67/3029 (2.2%) favored high-level amplification (score 2), and 277/3029 (9.1%) not favored (score 1). Kaplan–Meier survival analysis showed a significantly different overall survival among the groups (log-rank $p = .00016$; Figure 1A). In multivariate analysis adjusted for age and stage and stratified for the OTTA study, CCNE1 high-level amplified HGSC showed an HR of 1.26 (95% CI, 1.08–1.47) compared with the reference group with no evidence of amplification (Table 1). Data on the completeness of surgical cytoreduction were available for a subset of cases (66.9%) and, within this group, a sensitivity analysis

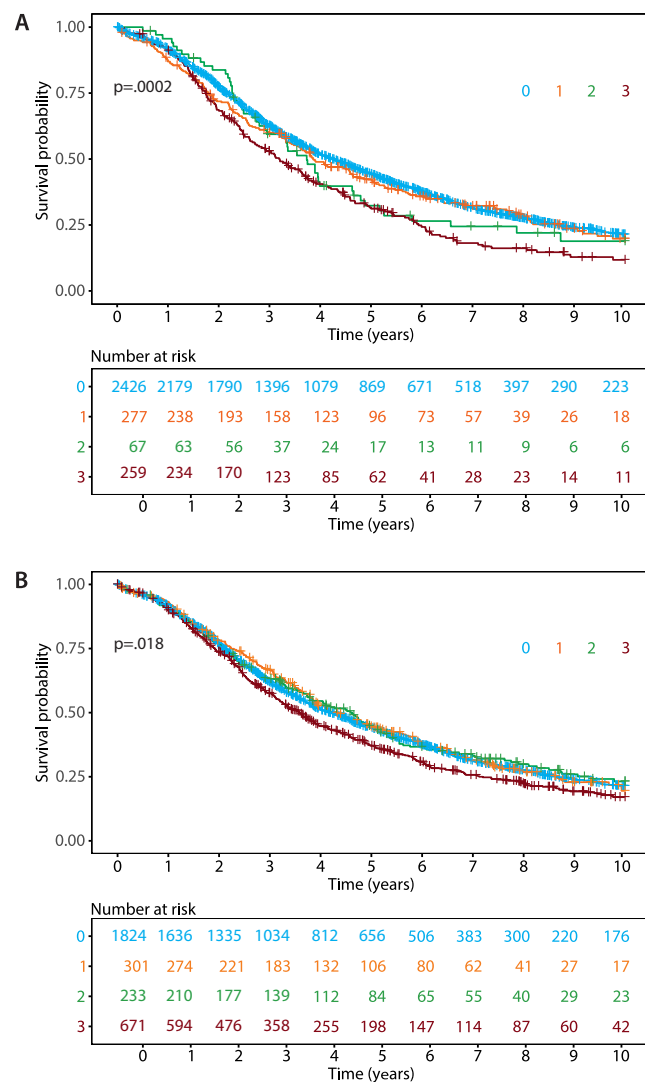


FIGURE 1 Kaplan–Meier overall survival analyses for (A) CISH score levels and (B) IHC score levels. CISH indicates chromogenic *in situ* hybridization; IHC, immunohistochemistry

adjusted for age, stage, completeness of surgical cytoreduction, and stratified for the OTTA study, resulted in the same HR of 1.27 (95% CI, 1.06–1.52; Table S2).

Prevalence of CCNE1 protein overexpression and association with overall survival

CCNE1 IHC showed overexpression (score 3) in 671/3029 (22.2%) cases and 1824/3029 (60.2%) had low CCNE1 protein levels (score 0) (Table 1). The remainder were equivocal, with 233/3029 (7.7%) favored to express high protein levels (score 2) and 301/3029 (9.9%) favored to express low levels (score 1). Kaplan–Meier survival analysis showed a significantly different survival between the groups (log-rank $p = .021$; Figure 1B). In multivariate analysis adjusted for age and stage, and stratified for OTTA study, CCNE1 high protein level HGSC showed an HR of 1.18 (95% CI, 1.05–1.32) compared with the group with low CCNE1 protein levels (Table 1). In a sensitivity multivariate analysis adjusted for age, stage, completeness of surgical cytoreduction, and stratified for OTTA study, a similar HR of 1.20 (95% CI, 1.05–1.37) was obtained (Table S2).

Associations of combined CCNE1 high-level amplification and protein level with overall survival

After binarization of CISH and IHC scores into scores 0/1 versus scores 2/3, 265/326 (81.3%) of high-level amplified cases showed high CCNE1 protein levels and, conversely, 2064/2703 (76.4%) of non-high-level amplified cases showed low CCNE1 protein levels. We then combined CCNE1 CISH and IHC into four groups (Figure 2, Table S3): first, negative for CCNE1 high-level amplification with low CCNE1 protein expression (CCNE1^{nonamp_lo}) comprising 68.1% (2064/3029) of the cases; second, negative for CCNE1 high-level amplification but with CCNE1 protein overexpression (CCNE1^{nonamp_hi}) comprising 21.1% (639/3029); third, CCNE1 high-level amplification but low CCNE1 protein expression (CCNE1^{amp_lo}) comprising 2.0% (61/3029); and fourth, CCNE1 high-level amplification with CCNE1 protein overexpression (CCNE1^{amp_hi}) comprising 8.8% (265/3029) of cases (Table 1). Kaplan–Meier survival analysis showed a significantly different overall survival among the groups (log-rank $p < .0001$, Figure 2). Patients in the CCNE1^{amp_hi} group had a 5-year survival rate of 28.3% compared with 41.9% in the CCNE1^{nonamp_lo} group (Table 1). This difference remained significant in multivariate modeling, following adjustment for age and stage and stratified for the OTTA study. The CCNE1^{amp_high} group had a higher risk of death (HR, 1.26; 95% CI, 1.09–1.47) compared with the reference CCNE1^{nonamp_lo} group (Table 1). In a sensitivity analysis adjusted for age, stage, completeness of surgical cytoreduction, and stratified for the OTTA study, the hazard ratio for the CCNE1^{amp_high} group compared with the reference CCNE1^{nonamp_lo} group was 1.20 (95% CI, 1.00–1.43; Table S2).

TABLE 1 Multivariate association between the expression and amplification of CCNE1 and overall survival in high-grade serous ovarian carcinoma ($n = 3029$)

CCNE1 profile	No. ^a	5-year survival (% ± SE)	Hazard ratio (95% CI) ^b	<i>p</i>
CCNE1 CISH score 0	2426	41.9 ± 1.1	Referent	.034 ^c
CCNE1 CISH score 1	277	40.2 ± 3.1	0.98 (0.84–1.14)	
CCNE1 CISH score 2	67	32.1 ± 6.2	0.97 (0.72–1.31)	
CCNE1 CISH score 3	259	29.5 ± 3.0	1.26 (1.08–1.47)*	
CCNE1 IHC score 0	1824	41.6 ± 1.2	Referent	.015 ^c
CCNE1 IHC score 1	301	43.0 ± 3.0	0.99 (0.85–1.16)	
CCNE1 IHC score 2	233	42.8 ± 3.4	0.92 (0.78–1.10)	
CCNE1 IHC score 3	671	35.4 ± 2.0	1.18 (1.05–1.32)*	
CCNE1 ^{nonamp_lo}	2064	41.9 ± 1.2	Referent	.033 ^c
CCNE1 ^{nonamp_hi}	639	41.0 ± 2.1	1.04 (0.93–1.16)	
CCNE1 ^{amp_lo}	61	37.8 ± 6.6	0.97 (0.71–1.34)	
CCNE1 ^{amp_high}	265	28.3 ± 3.0	1.26 (1.09–1.47) ^c	

Abbreviations: CCNE1, cyclin E1; CCNE1^{amp}, CCNE1 high-level amplification; CCNE1^{hi}, CCNE1 protein overexpression by immunohistochemistry; CCNE1^{lo}, negative for CCNE1 protein overexpression by immunohistochemistry; CCNE1^{nonamp}, negative for CCNE1 high-level amplification; CISH, chromogenic in situ hybridization; HGSC, high-grade serous ovarian carcinoma; HR, hazard ratio; IHC, immunohistochemistry; OS, overall survival.

^aThe same cohort was assessed in univariate survival analysis.

^bHR adjusted for patient age and stage, with stratification by the Ovarian Tissue Tumor Analysis study; Cox proportional regression modeling was used to calculate *p* values and define significance.

^cStatistically significant values.

* $p < .05$.

Associations of combined CCNE1 high-level amplification and protein expression with clinical parameters and biomarkers in HGSC

The univariate associations of the combined groups with clinicopathological parameters are shown in Table 2. Patients diagnosed with CCNE1 high-level amplified HGSC were older, with a trend toward a higher likelihood of residual disease after debulking surgery. No associations were observed for stage (International Federation of Gynecology and Obstetrics I, II [locoregional] compared with International Federation of Gynecology and Obstetrics III/IV [distant]) or the timing of the primary chemotherapy regimen (adjuvant vs. neo-adjuvant; Table S4). For subsets with available data, the four groups showed significant associations with TP53 IHC (available data for 65.9% of cases), BRCA1/2 germline variant (available data for 31.5% of cases), CDKN2A IHC (available data for 64.2% of cases), and RB1 IHC status (available data for 71.1% of cases; Table 3). Normal TP53 IHC was most prevalent in the CCNE1^{nonamp_lo} group. However, the abnormal TP53 IHC patterns, which are surrogates for the functional groups of TP53 mutations,³¹ were not different. Germline BRCA1/2 mutations were rarely present in CCNE1 high-level amplified HGSC. Only two HGSC cases had protein-truncating deleterious BRCA2 variants and both cases had a CCNE1 CISH score of 2 (equivocal favor high). The CCNE1^{amp_high} group had the highest frequency of CDKN2A block expression, a surrogate for RB pathway activation, but there were no cases with complete absence of CDKN2A

expression, a surrogate for a deleterious deletion of CDKN2A. CCNE1 high-level amplification was inversely associated with loss of RB1.

CCNE1 mRNA expression by NanoString in HGSC

For 1612/3029 overlapping cases with CCNE1 mRNA expression, there was moderate correlation between CCNE1 mRNA expression and CISH scores ($r = 0.478$) and CCNE1 IHC scores ($r = 0.544$; Figure 3). CCNE1 mRNA expression was also different across the four combined groups, with the highest level observed in CCNE1^{amp_high}, followed by CCNE1^{amp_lo} and CCNE1^{nonamp_hi} (Figure 3). Lastly, we evaluated the associations of CCNE1 mRNA expression with overall survival in 2419 HGSC cases. The clinicopathological characteristics of these cases are shown in Table S5. When considering a 1-SD increase in CCNE1 mRNA expression score, there was no association with overall survival (HR, 1.00; 95% CI, 0.94–1.06, $p = .96$; Table S6). This also was the case when using a cutoff at the top 10% versus the remainder (HR, 1.06; 95% CI, 0.88–1.27, $p = .53$; Table S6).

DISCUSSION

In this study, we validate the association of combined CCNE1 high-level gene amplification and CCNE1 protein overexpression with overall survival in a large cohort of patients with HGSC from the

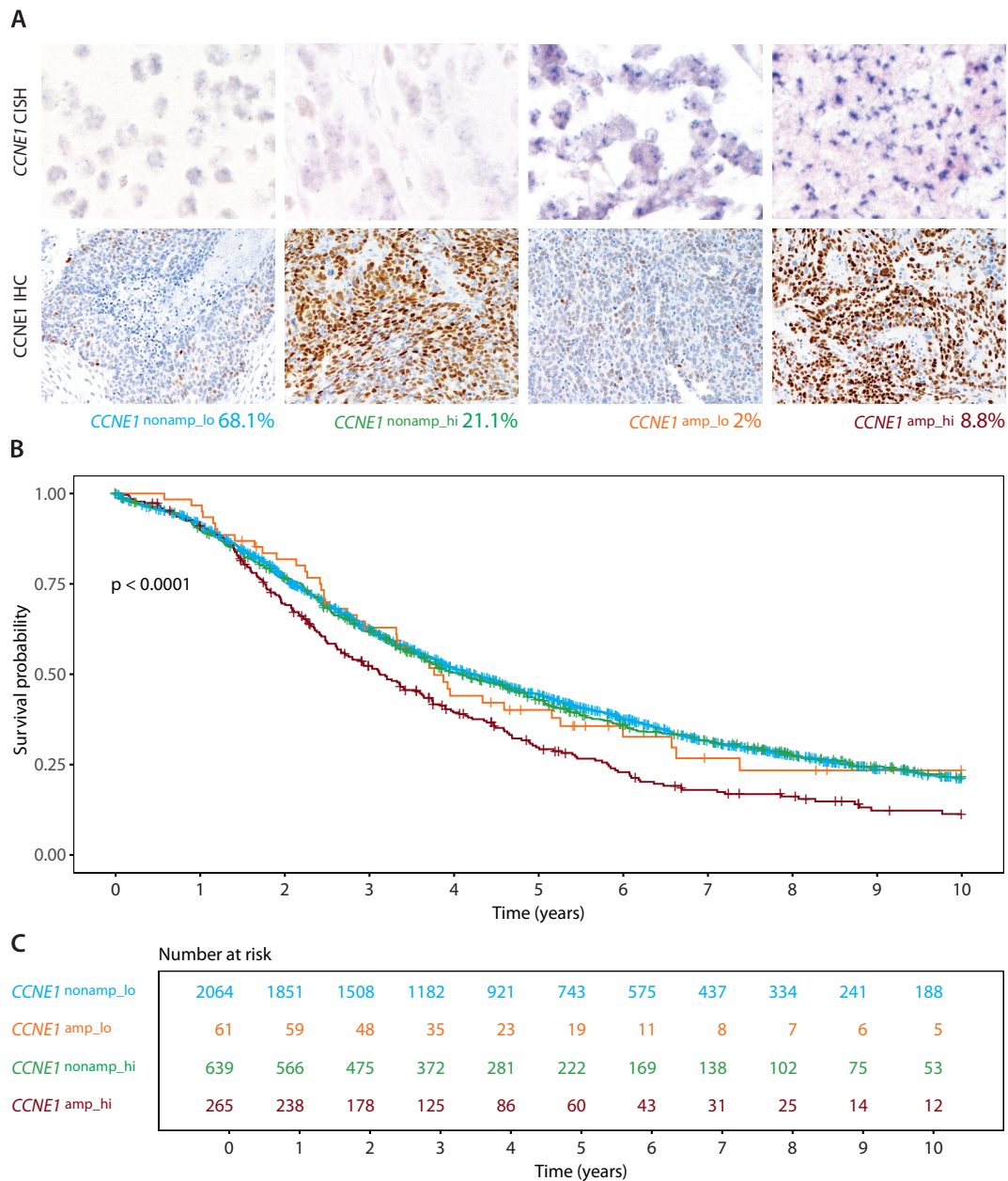


FIGURE 2 (A) CCNE1 DNA CISH and IHC combinations resulting in four groups: CCNE1^{nonamp_lo} CISH showing no high-level amplification and IHC <60% positive tumor cell nuclei, CCNE1^{nonamp_hi} CISH showing no high-level amplification and IHC >60% positive and >5% strongly staining tumor cell nuclei, CCNE1^{amp_lo} CISH showing high-level amplification and IHC <60% positive tumor cell nuclei, CCNE1^{amp_hi} CISH showing high-level amplification, and IHC >60% positive and >5% strongly staining tumor cell nuclei. (B) Kaplan-Meier overall survival analysis for four combined CISH/IHC groups. (C) Risk table indicating the number of patients within the cohort that are at risk of death, observed at a yearly. CISH, chromogenic *in situ* hybridization; IHC, immunohistochemistry

OTTA consortium. Our results demonstrate that assessing CCNE1 at a DNA copy-number level and protein level is a more robust determinant of prognosis than mRNA expression. We also confirm that CCNE1 high-level amplification is essentially mutually exclusive with pathogenic *BRCA1/2* germline alterations and associated with biomarker changes in the *RB1* pathway.

For the association of CCNE1 protein expression with survival, the genomic context seems to matter. The fairly large group of CCNE1^{nonamp_hi} shows a similar survival compared with the

CCNE1^{nonamp_lo} reference but longer survival relative to the CCNE1^{amp_hi} group. Both the CCNE1^{nonamp_hi} and CCNE1^{amp_hi} group express similarly high protein levels but CCNE1^{amp_hi} express higher mRNA levels than CCNE1^{nonamp_hi}, suggesting that amplification-driven CCNE1 overexpression is due to higher transcriptional activity, whereas CCNE1 overexpression in CCNE1^{nonamp_hi} cases may be more dependent on protein stabilization or lack of degradation.³² We, however, demonstrate that differences in CCNE1 mRNA expression were not associated with overall survival in HGSC. We

TABLE 2 Clinicopathological parameters by combined CCNE1 protein and amplification status ($n = 3029$)

Clinicopathological variable	CCNE1 profile				p^a	Total
	CCNE1 ^{nonamp_lo}	CCNE1 ^{nonamp_hi}	CCNE1 ^{amp_lo}	CCNE1 ^{amp_hi}		
Number of cases, n (%) ^b	2064 (68.1)	639 (21.1)	61 (2.0)	265 (8.8)		3029 (100.0)
Age at diagnosis, years						
Mean \pm SD	60.9 \pm 11.4	61.7 \pm 10.9	65.0 \pm 9.11	65.0 \pm 9.8		61.5 \pm 11.2
Median	61	62	66	65		62
Range	21–93	30–92	40–84	38–91		21–93
Stage, n (%) ^c					.3848	
FIGO I, II (locoregional)	350 (17.0)	124 (19.4)	9 (14.8)	41 (15.5)		525 (17.3)
FIGO III, IV (distant)	1714 (83.0)	515 (80.6)	52 (85.2)	224 (84.5)		2527 (82.7)
Completeness of survival cytoreduction					.0563 ^d	
No residual disease, n (%) ^c	555 (40.7)	200 (44.5)	10 (34.5)	61 (33.2)		826 (40.8)
Residual disease present, n (%) ^c	809 (59.3)	249 (55.5)	19 (65.5)	123 (66.9)		1200 (59.2)
Unknown, n ^c	700	190	32	81		1003

Abbreviations: CCNE1, cyclin E1; CCNE1^{amp}, CCNE1 high-level amplification; CCNE1^{hi}, CCNE1 protein overexpression by immunohistochemistry; CCNE1^{lo}, negative for CCNE1 protein overexpression by immunohistochemistry; CCNE1^{nonamp}, negative for CCNE1 high-level amplification; FIGO, International Federation of Gynecology and Obstetrics.

^aChi-square testing was used to calculate p values. Statistically significant values shown; $p < .05$.

^bThe proportion of cases in each score stratum is given as a percentage of the total patients examined.

^cThe proportion of cases is given as a percentage of the total cases within each score stratum.

^dChi-square testing to compare the proportions of cases with absent vs. present residual disease status. This does not include cases in which residual disease status was unknown.

obtained consistent HRs close to 1.0 by studying 2419 samples in the current study, confirming the results from a previous OTTA study.¹⁹ Despite the strong correlation of mRNA with amplification status and protein levels, the lack of survival association may be caused by dilution of the mRNA signal in tumor bulk analysis with varying tumor cellularity compared with the spatially controlled CISH and IHC assays. The survival differences of the two groups with high CCNE1 protein levels still creates a conceptual dilemma because it is the protein that is exerting the function, and the mechanism of protein accumulation should not matter, unless there is a difference in the timing of expression in relation to the cell cycle or the functional quality of CCNE1 protein. In high CCNE1-expressing breast cancer, CCNE1 can be proteolytically cleaved into low-molecular weight derivatives.³³ An alternative explanation might be that in the CCNE1^{amp_hi} group, other oncogenes coamplified with CCNE1 on 19q12, such as *URI* contribute to survival.³⁴

Although we confirm that the group of CCNE1^{amp_hi} is associated with the shortest overall survival, we also show that this association is mainly driven by DNA copy-number status, achieving a better stratification than protein level. However, protein level seems to provide additional information by singling out the small group of CCNE1^{amp_lo}, which in the main analysis had a similar HR compared with the reference CCNE1^{nonamp_lo}. In a sensitivity analysis including residual tumor, the HR was more similar to the high-risk group CCNE1^{amp_hi}. However, this was not statistically significant with the small case numbers in the CCNE1^{nonamp_lo} subgroup, and this

sensitivity analysis may have introduced bias for the small subgroups that are not comparable to the overall cohort. This raises a question about the importance of the level of CCNE1 protein expression in the context of CCNE1 high-level amplification. Both CCNE1^{amp_hi} and CCNE1^{amp_lo} groups are similar regarding clinical parameters (i.e., age, residual disease) and rarely harbored *BRCA1/2* germline alterations; loss of *RB1* was uncommon. Although this suggests no difference and assessment of the DNA copy number status would be sufficient, both groups differed in regard to the abnormal block *CDKN2A* expression status, which was highest in CCNE1^{amp_hi}, indicating a higher *RB1* pathway dependent on the CCNE1 protein level. Based on our observed differences in survival and CCNE1 mRNA expression, together with previous study findings,^{15,23} we interpret that CCNE1^{amp_hi} is different from CCNE1^{amp_lo}. By focusing on the CCNE1^{amp_hi} group, IHC can be used to screen HGSC samples for CCNE1 overexpression followed by copy-number assessment for clinical trial inclusion, which would pragmatically circumvent the limited sensitivity of CCNE1 IHC. However, the clinical significance of this relatively small group remains uncertain. We cannot entirely exclude a misclassification based on the CISH or IHC assay. Future studies should use full-section IHC to exclude potential intratumoral heterogeneity of the protein expression and alternative copy-number assays for the small group of CCNE1^{amp_lo} tumors. However, some CCNE1 high-level amplified tumors may not express high protein levels. The Cancer Genome Atlas reported that low *CDKN2A* mRNA expression is mutually exclusive with CCNE1

TABLE 3 Univariable associations with selected biomarkers by combined CCNE1 protein and amplification status ($n = 3029$)

Molecular marker ^b	Status	CCNE1 profile ^a				p^c	Total ^d
		CCNE1 ^{nonamp_lo}	CCNE1 ^{nonamp_hi}	CCNE1 ^{amp_lo}	CCNE1 ^{amp_hi}		
TP53	Abnormal	1202 (89.9)	413 (94.3)	42 (100.0)	172 (95.6)	.0008 ^e	1829 (91.6)
	Normal	135 (10.1)	25 (5.7)	0 (0.0)	8 (4.4)		168 (8.4)
	Unknown	727	201	19	85		1032
Abnormal TP53 IHC patterns ^f	Abnormal OE	830 (69.1)	296 (71.7)	31 (73.8)	120 (69.8)	.8706	1277 (69.8)
	Abnormal CA	311 (25.9)	98 (23.7)	10 (23.8)	46 (26.7)		465 (25.4)
	Abnormal CY	61 (5.1)	19 (4.6)	1 (2.4)	6 (3.5)		87 (4.8)
BRCA1/2 germline pathogenic variant	Present	111 (16.9)	33 (16.5)	0 (0.0)	2 (2.9)	.0020 ^e	146 (15.3)
	Absent	546 (83.1)	167 (83.5)	28 (100.0)	67 (97.1)		808 (84.7)
	Unknown	1407	439	33	196		2075
CDKN2A	Normal	630 (48.2)	125 (29.7)	12 (30.8)	33 (18.6)	<.0001 ^e	800 (41.2)
	Abnormal block positive	591 (45.2)	288 (68.4)	24 (61.5)	144 (81.4)		1047 (53.9)
	Abnormal complete absence	86 (6.6)	8 (1.9)	3 (7.7)	0 (0.0)		97 (5.0)
	Unknown	757	218	22	88		1085
RB1	Normal (retained)	1153 (81.1)	402 (83.6)	44 (97.8)	187 (91.2)	.0001 ^e	1786 (83.0)
	Abnormal (loss)	269 (18.9)	79 (16.4)	1 (2.2)	18 (8.8)		367 (17.0)
	Unknown	642	158	16	60		876
Total ^c		2064 (68.1)	639 (21.1)	61 (2.0)	265 (8.8)		3029 (100.0)

Abbreviations: CA, complete absence; CCNE1, cyclin E1; CCNE1^{amp}, CCNE1 high-level amplification; CCNE1^{hi}, CCNE1 protein overexpression by immunohistochemistry; CCNE1^{lo}, negative for CCNE1 protein overexpression by immunohistochemistry; CCNE1^{nonamp}, negative for CCNE1 high-level amplification; CY, cytoplasmic; IHC, immunohistochemistry; OE, overexpression.

^aCCNE1 profile amplification is defined by chromogenic *in situ* hybridization and, and protein expression is defined by immunohistochemistry.

^bThe proportion of cases with a particular molecular marker status is given as a percentage of the total patients examined in each CCNE1 profile. This does not include cases where mutational status was unknown.

^cThe proportion of cases in each CCNE1 profile is given as a percentage of the total patients examined. This does not include cases where mutational status was unknown.

^dChi-square testing was used to calculate p values. This does not include cases where mutational status was unknown.

^eStatistically significant values; $p < .05$.

^fTP53 type of abnormal mutation-type immunohistochemical pattern: OE, CA, and CY.

amplification.¹¹ We observed a small number of cases with loss of CDKN2A protein (a surrogate for CDKN2A deep deletions) in the CCNE1^{amp_lo} but not in the CCNE1^{amp_hi}, suggesting that another concurrent RB1 pathway alteration could prevent CCNE1 protein overexpression in the context of CCNE1 high-level DNA amplifications.

Our results confirm that CCNE1 high-level DNA amplifications are essentially mutually exclusive with pathogenic BRCA1/2 germline alterations. The two exceptional cases with pathogenic BRCA2 germline variants that were grouped as CCNE1^{amp_hi} were scored as equivocal favor high by CISH. These rare cases of “double classifiers” may require additional assays such as validated homologous recombination deficiency assays or copy number signatures to assign as homologous recombination-deficient or homologous recombination-proficient. From a treatment perspective, the CCNE1^{amp_hi} group had a shorter survival likely in part because of lower response to

platinum-based chemotherapy, which correlates with insensitivity to PARP inhibitors. Therefore, the CCNE1^{amp_hi} group may not respond to PARP inhibitors, making CCNE1^{amp_hi} a candidate biomarker that could be used as a negative predictive test for PARP inhibitors. This hypothesis could be tested in secondary analyses of clinical trials that include unselected HGSC patients treated with PARP inhibitors.¹⁸

Novel treatment approaches are required for women diagnosed with CCNE1^{amp_hi} HGSC.³⁵ Bowtell and colleagues observed decreased tumorigenesis in CDK2-knockout HGSC cell lines with CCNE1 amplifications. However, the CDK2 inhibitor, dinaciclib, did not suppress tumorigenesis, probably because it is not entirely specific for CDK2.³⁶ Perhaps a more specific CDK2 inhibitor could be tested on patients with HGSC and CCNE1^{amp_hi} HGSC. It remains to be seen whether redundancies in the CDK2/CCNE1 pathway (CDK1 for CDK2, CCNE2 for CCNE1) observed in normal cells pose another challenge of targeting this pathway in cancers.^{3,37} In a post hoc

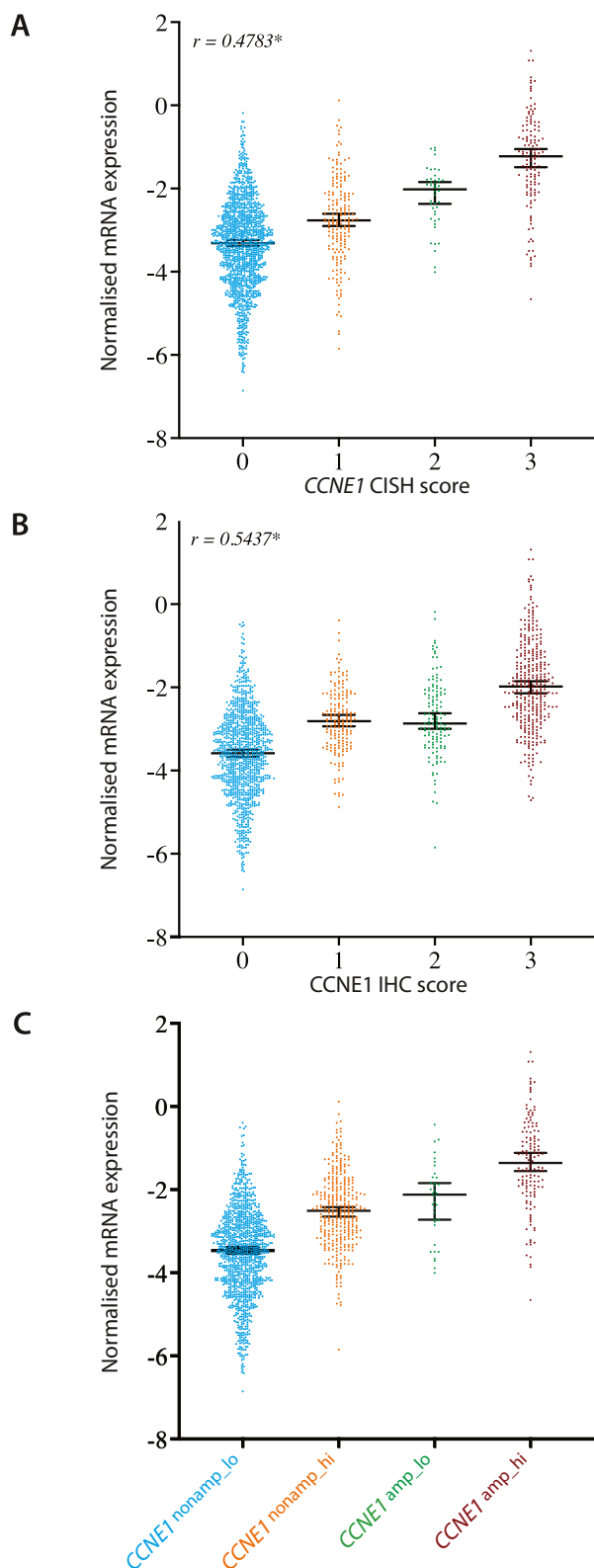


FIGURE 3 (A) Correlation of CCNE1 DNA CISH score with normalized mRNA expression. (B) Correlation of CCNE1 protein IHC score with normalized RNA expression. (C) Association of four combined CISH/IHC groups with normalized RNA expression. Pearson's correlation analysis given by r . * $p < .05$. CCNE1 indicates cyclin E1; CISH, chromogenic *in situ* hybridization; IHC, immunohistochemistry

analysis of a clinical trial investigating the Wee1 inhibitor adavosertib in combination with gemcitabine, CCNE1-amplified tumors were more likely to respond.³⁸ Through phosphorylation of the CDK1/CCNB complex, Wee1 kinase is an inhibitor of the G2/M transition, which is more critical for HGSC with deficient G1/S transitions. Notably, in a recent phase 2 trial, adavosertib has also shown promising response rates in CCNE1 overexpressing recurrent HGSC regardless of amplification status.³⁹ Alternatively, using a CRISPR-Cas9-screen, *PKMYT1*, which encodes a protein kinase also involved in G2/M transition, was identified as a synthetic lethal target for CCNE1 high-expressing cells, which were sensitive to inhibition by a selective *PKMYT1* inhibitor.⁴⁰ This suggests that perhaps both CCNE1 expression and amplification status should be assessed when testing CCNE1 as predictive marker for new molecular therapy.

Although the main function of CCNE1 is in cell-cycle progression, the main oncogenic effect may be independent from proliferation. High proliferating HGSCs are associated with longer survival, likely because of better response to standard chemotherapy,^{27,28} whereas CCNE1 alterations are associated with shorter survival and poor response to chemotherapy. CCNE1 protein expression is only weakly correlated with proliferation markers (Ki67, minichromosome maintenance complex component 3).²⁸ Although uncontrolled cell-cycle entry remains the main known function of CCNE1, overall, these data suggest that much of CCNE1's oncogenic function is related to a premature S-phase entry resulting in chromosomal instability rather than increased proliferation.^{7,8}

The main limitation of our study was the assay resolution. We did not count the DNA copy number ratio by using a CEP19 control probe but focused on the presence of CCNE1 clusters as a surrogate for high-level amplification defined by >8 copy numbers, which was previously orthogonally validated using the NanoString CNV assay and digital polymerase chain reaction.¹⁵ Not using ratios prevented us from assessing low-level gains. The prevalence of CCNE1 high-level amplifications is approximately half compared with previous studies reporting CCNE1 amplification (frequency of 20%), which is due to the higher cutoff we used.^{11,12} Our present study used CISH analysis, which is a well-established and clinically adopted technique to interrogate genetic amplification such as evaluation of *ERBB2* amplification in breast and gastric cancer. However, next-generation sequencing (NGS)-based assays such as whole-genome/exome sequencing or targeted panel sequencing are being increasingly used in the clinical setting to provide more comprehensive molecular characterization of tumors, including copy number alterations. In contrast to CISH (or fluorescence *in situ* hybridization) assays that provide spatially focused analysis that evaluates signals only from carcinoma cells, the NGS-based assays typically use bulk tumor samples in which tumor content can vary, and it may have lower sensitivity compared with spatially controlled assays such as *in situ* hybridization, particularly from samples with low tumor content in the settings of core needle biopsies or posttreatment (neoadjuvant chemotherapy) samples. Another consideration is that CISH analysis generally requires less amount of tumor tissue than NGS-based analysis, which may be relevant in cases

in which a limited amount of diagnostic tissue is available. Future studies are required to determine the clinical utility and limitations of NGS-based assay for *CCNE1* copy number evaluation. There were limited data annotations for some analyses because of missing data for residual disease and germline *BRCA1/2* status.

In conclusion, our large-scale validation with survival data supports the notion that *CCNE1* is the most promising biomarker to define the largest subgroup of homologous recombination-proficient HGSC. *CCNE1* high-level amplifications should be studied as negative predictive markers for current standard therapies (chemotherapy, PARP inhibitors) and should be evaluated in clinical trials assessing novel treatment approaches. We propose to focus initially on the *CCNE1*^{amp,hi} group; *CCNE1* IHC could be used as a screening tool, followed by an assessment of DNA copy number status.

ACKNOWLEDGMENTS

The study was funded by Alberta Precision Laboratory research support fund (RS17-601, RS10-526). This work was funded by the National Institutes of Health/National Cancer Institute (NCI) grants to S. J. Ramus (grant number R01CA172404). The Australian Ovarian Cancer Study (AOCS) was supported by the U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0729, The Cancer Council Victoria, Queensland Cancer Fund, The Cancer Council New South Wales, The Cancer Council South Australia, The Cancer Foundation of Western Australia, The Cancer Council Tasmania and the National Health and Medical Research Council of Australia (NHMRC; ID199600; ID400413 and ID400281). The AOCS gratefully acknowledges additional support from the Peter MacCallum Foundation and Ovarian Cancer Australia. BGS: Breast Cancer Now and the Institute of Cancer Research (ICR). ICR acknowledges NHS funding to the NIHR Biomedical Research Centre. BRZ: Brazilian National Council for Scientific and Technological Development, grant No. 478416/2009-1; CAL: Cancer Research Society (19319) and CLS Internal Research support RS14-508; CNI: CNI funded by Instituto de Salud Carlos Tercero (AES grant PI19/01730) and Fondo Europeo de Desarrollo Regional, FEDER. Instituto de Salud Carlos III (PI 12/01319); Ministerio de Economía y Competitividad (SAF2012); HOP: Department of Defense (DAMD17-02-1-0669) and NCI (K07-CA080668, R01-CA95023, MO1-RR000056). This project used the UPMC Hillman Cancer Center and Tissue and Research Pathology/Pitt Biospecimen Core shared resource which is supported in part by award P30CA047904. LAX: American Cancer Society Early Detection Professorship (SIOP-06-258-01-COUN) and the National Center for Advancing Translational Sciences (NCATS), Grant UL1TR000124; MAY: National Institutes of Health (R01-CA122443, R01-CA243483, P30-CA15083, P50-CA136393); Mayo Foundation; Minnesota Ovarian Cancer Alliance; Fred C. and Katherine B. Andersen Foundation; NCT: Parts of the study at the NCT were funded by "Heuer Stiftung für medizinische Forschung"; POC: Pomeranian Medical University; SEA: Cancer Research UK C490/A16561, the UK National Institute for Health Research Biomedical Research Centres at the University of Cambridge, Cambridge Cancer Centre. The University of Cambridge has received salary support for Paul D.P. Pharoah from the

NHS in the East of England through the Clinical Academic Reserve. TVA: This work was supported by Canadian Institutes of Health Research grant (MOP-86727) and by NIH/NCI 1 R01CA160669-01A1; UKO: The UKOPS study was funded by The Eve Appeal (The Oak Foundation) with contribution to author's salary through MRC core funding (MC_UU_00004/01) and the National Institute for Health Research University College London Hospitals Biomedical Research Centre; VAN: BC's Gynecological Cancer Research Team (OVCARE) receives core funding from The BC Cancer Foundation and the VGH and UBC Hospital Foundation; WMH: National Health and Medical Research Council of Australia, Enabling Grants ID 310670 and ID 628903. Cancer Institute NSW Grants 12/RIG/1-17 and 15/RIG/1-16. The Westmead GynBiobank acknowledges financial support from the Sydney West Translational Cancer Research Centre, funded by the Cancer Institute NSW; COE: The GYN-COE program, T.P. Conrads, Y. Cassablanca, G.L. Maxwell, and K.M. Darcy are funded by the U.S. Defense Health Program (grants HU0001-16-2-0006 and HU0001-19-2-0031); The Health Science Alliance (HAS) Biobank, UNSW Sydney, Australia, is funded by the Translational Cancer Research Network (TCRN), a Translational Cancer Research Centre supported by the Cancer Institute NSW. The SWE cohort is funded by the Swedish Cancer Foundation (CAN 2018/384).

Funding for authors:

S. Ramus is supported by National Health and Medical Research Council of Australia (NHMRC) grant APP2009840. N.S. Meagher is supported by the NSW Ministry of Health and UNSW Sydney under the NSW Health PhD Scholarship Program, and the Translational Cancer Research Network, a translational cancer research center program funded by the Cancer Institute NSW. J. Millstein is funded by NCI grant P30CA014089. S. Heublein was funded by Heuer Stiftung für medizinische Forschung. M.S. Anglesio is funded through a Michael Smith Foundation for Health Research Scholar Award and the Janet D. Cottrelle Foundation Scholars program managed by the BC Cancer Foundation. BC's Gynecological Cancer Research team (OVCARE) receives support through the BC Cancer Foundation and The VGH+UBC Hospital Foundation. L. Loverix is predoctoral researcher of the Fund for Scientific Research-Flanders (FWO Vlaanderen 1S41921). T. Van Gorp is a Senior Clinical Investigator of the Fund for Scientific Research-Flanders (FWO Vlaanderen 18B2921N). The funding for K.M. Darcy and preparation of tumor samples and clinical samples are from awards HU0001-16-2-0006 and HU0001-21-2-0027 from the Uniformed Services University of the Health Sciences from the Defense Health Program to the Henry M Jackson Foundation (HJF) for the Advancement of Military Medicine Inc. Gynecologic Cancer Center of Excellence Program (PI: Yovanni Casablanca, Co-PI: G. Larry Maxwell). D. Khabele is supported by NIH NCI grants R01CA243511 and R21CA210210. A. Staebler received funding by Deutsche Forschungsgemeinschaft as part of the CRC 685. R.E. was supported by the Interdisciplinary Center for Clinical Research (IZKF; Clinician Scientist Program) of the Medical Faculty FAU Erlangen-Nürnberg. U. Menon and A. Gentry-Maharaj are supported by salary contributions through MRC core funding (MC_UU_00004/01).

For all participating studies, we thank all the women who participated in research, study staff, study participants, physicians, nurses, health care providers, and health information sources who have contributed to the study. We thank Shuhong Liu and Young Ou from the Anatomical Pathology Research Laboratory at the University of Calgary for performing immunohistochemistry and chromogenic in situ hybridization, and Thomas Kryton, image specialist, for compiling the composite figures. We thank the AOCS Study Group (<http://www.aocstudy.org>); see the supporting information. The contents of the published material are solely the responsibility of the authors and do not reflect the views of the National Health and Medical Research Council of Australia.

Open access publishing facilitated by University of New South Wales, as part of the Wiley - University of New South Wales agreement via the Council of Australian University Librarians.

CONFLICTS OF INTEREST

The authors have no relevant conflicts of interest regarding this publication. A. Hartmann has received honoraria from BMS, MSD, Roche, AstraZeneca, Boehringer Ingelheim, AbbVie, Jansen-Cilag, and Ipsen.










T.P. Conrads is member of the Thermo Fisher Scientific Inc scientific advisory board. P.A. Cohen is a member of the Clinic IQ Scientific Advisory Board and has received honoraria from Seqirus and AstraZeneca. D. Bowtell has research funding from AstraZeneca, Beigene, and Genentech Roche, and is an advisor to Exo Therapeutics. U. Menon has received an honorarium from the NY Obstetrical Society and held personal shares between 1 April 2011 and 30 October 2021. T. Van Gorp has received honoraria for advisory boards from Eisai Europe (to institute), OncXerna Therapeutics (to institute), AstraZeneca (to institute), GSK (to institute), MSD (to institute), research funding from Amgen (to institute), Roche (to institute), and AstraZeneca (to institute) and travel reimbursements from MSD, Immunogen, PharmaMar, and AstraZeneca. P. Harter has received honoraria from Amgen, AstraZeneca, GSK, Roche, Sotio, Stryker, Zai Lab, MSD, Clovis, and Eisai; has served on advisory boards for AstraZeneca, Roche, GSK, Clovis, Immunogen, MSD, and Eisai; and has received research funding (to institute) from AstraZeneca, Roche, GSK, Genmab, DFG, European Union, DKH, Immunogen, and Clovis. P. Ghatage has received honoraria from AstraZeneca, GSK, and Eisai. I. Vergote has received consulting fees from Agenus, Akesobio, AstraZeneca, Bristol-Myers Squibb, Deciphera Pharmaceuticals, Eisai, Elevar Therapeutics, F. Hoffmann-La Roche, Genmab, GSK, Immunogen, Jazzpharma, Karyopharm, Mersana, MSD, Novocure, Novartis, Oncoinvent, OncXerna, Sanofi, Seagen, Sotio, Verastem Oncology, and Zentalis, done contracted research with Oncoinvent AS and corporate sponsored research with Amgen and Roche, and received travel expenses/accommodations from Karyopharm and Genmab. D. Bowtell has received honoraria from AstraZeneca, has a consulting role with Exo Therapeutics Research and receives funding from Roche/Genentech, AstraZeneca and BeiGene. A. DeFazio received grant funding from AstraZeneca. A.H. had an advisory role and received honoraria from BMS, MSD, Roche, Cepheid, Qiagen, Agilent, Diaceutics, Lilly, AstraZeneca, Boehringer Ingelheim, AbbVie, Jansen-Cilag, Pfizer, and Ipsen.

R. Erber has received honoraria from Roche, Eisai, Pfizer, BioNTech, Veracyte (PROCURE), Diaceutics, and Novartis. The institution of A. Hartmann and R. Erber conducts research for AstraZeneca, Roche, Jansen-Cilag, NanoString Technologies, Biocartis, ZytoVision, Novartis, Cepheid, and BioNTech. S. Heublein received honoraria from Clovis, Merck Sharp & Dohme Corporation and Pfizer, and has received research funding from Neuer Stiftung fuer Medizinische Forschung and Novartis as well as travel support from GlaxoSmithKline.

AUTHOR CONTRIBUTIONS

Eun-Young Kang: Immunohistochemical protein and chromogen in situ hybridization scores collection and manuscript draft and revision. **Ashley Weir:** Analyses. **Kyo Farrington:** Immunohistochemical protein and chromogen in situ hybridization scores collection. **Paul D.P. Pharoah:** Statistical advice. **Susan J. Ramus:** Study conception, design, and supervision. **Martin Koebel:** Study conception, design, and supervision; immunohistochemical protein and chromogen in situ hybridization scores collection; and manuscript draft and revision. All authors contributed through collection, curation, and maintenance of respective consortia-based, or local institution, collections of patient samples including recruitment and consenting of patients, clinical care, abstraction of clinical data, and updating of outcome and follow-up data. All authors revised the manuscript and approved submission of the final version.

ORCID

Nicola S. Meagher  <https://orcid.org/0000-0001-9134-2118>
 Cheng-Han Lee  <https://orcid.org/0000-0003-2094-1222>
 Sian Fereday  <https://orcid.org/0000-0002-8559-8579>
 Catherine J. Kennedy  <https://orcid.org/0000-0002-4465-5784>
 Naoko Sasamoto  <https://orcid.org/0000-0002-4526-2181>
 Joellen M. Schildkraut  <https://orcid.org/0000-0002-0990-9339>
 Aline Talhouk  <https://orcid.org/0000-0001-7760-410X>
 Ignace Vergote  <https://orcid.org/0000-0002-7589-8981>
 Martin Köbel  <https://orcid.org/0000-0002-6615-2037>

REFERENCES

1. Kanska J, Zakhour M, Taylor-Harding B, Karlan BY, Wiedemeyer WR. Cyclin E as a potential therapeutic target in high grade serous ovarian cancer. *Gynecol Oncol*. 2016;143(1):152-158. doi:10.1016/j.ygyno.2016.07.111
2. Gorski JW, Ueland FR, Kolesar JM. CCNE1 amplification as a predictive biomarker of chemotherapy resistance in epithelial ovarian cancer. *Diagnostics*. 2020;10(5):279. doi:10.3390/diagnostics10050279
3. Caldon CE, Musgrove EA. Distinct and redundant functions of cyclin E1 and cyclin E2 in development and cancer. *Cell Div*. 2010;5(1):2. doi:10.1186/1747-1028-5-2
4. Lacey KR, Jackson PK, Stearns T. Cyclin-dependent kinase control of centrosome duplication. *Proc National Acad Sci USA*. 1999;96(6):2817-2822. doi:10.1073/pnas.96.6.2817
5. Koff A, Giordano A, Desai D, et al. Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. *Science*. 1992;257(5077):1689-1694. doi:10.1126/science.1388288
6. Spruck CH, Won KA, Reed SI. Deregulated cyclin E induces chromosome instability. *Nature*. 1999;401(6750):297-300. doi:10.1038/45836

7. Bester AC, Roniger M, Oren YS, et al. Nucleotide deficiency promotes genomic instability in early stages of cancer development. *Cell*. 2011;145(3):435-446. doi:10.1016/j.cell.2011.03.044
8. Karst AM, Jones PM, Vena N, et al. Cyclin E1 deregulation occurs early in secretory cell transformation to promote formation of fallopian tube-derived high-grade serous ovarian cancers. *Cancer Res*. 2014;74(4):1141-1152. doi:10.1158/0008-5472.can-13-2247
9. Etemadmoghadam D, de Fazio A, Beroukhi R, et al. Integrated genome-wide DNA copy number and expression analysis identifies distinct mechanisms of primary chemoresistance in ovarian carcinomas. *Clin Cancer Res*. 2009;15(4):1417-1427. doi:10.1158/1078-0432.ccr-08-1564
10. Nakayama N, Nakayama K, Shamima Y, et al. Gene amplification CCNE1 is related to poor survival and potential therapeutic target in ovarian cancer. *Cancer*. 2010;116:2621-2634. doi:10.1002/cncr.24987
11. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011;474(7353):609-615. doi:10.1038/nature10166
12. Stronach EA, Paul J, Timms KM, et al. Biomarker assessment of HR deficiency, tumor BRCA1/2 mutations, and CCNE1 copy number in ovarian cancer: associations with clinical outcome following platinum monotherapy. *Mol Cancer Res*. 2018;16(7):1103-1111. doi:10.1158/1541-7786.mcr-18-0034
13. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2(5):401-404. doi:10.1158/2159-8290.cd-12-0095
14. Etemadmoghadam D, Weir BA, Au-Yeung G, et al. Synthetic lethality between CCNE1 amplification and loss of BRCA1. *Proc National Acad Sci USA*. 2013;110(48):19489-19494. doi:10.1073/pnas.1314302110
15. Chan AM, Enwere E, McIntyre JB, et al. Combined CCNE1 high-level amplification and overexpression is associated with unfavourable outcome in tubo-ovarian high-grade serous carcinoma. *J Pathol Clin Res*. 2020;6(4):252-262. doi:10.1002/cjp2.168
16. Macintyre G, Goranova TE, De Silva D, et al. Copy number signatures and mutational processes in ovarian carcinoma. *Nat Genet*. 2018;50(9):1262-1270. doi:10.1038/s41588-018-0179-8
17. Vázquez-García I, Uhlitz F, Ceglia N, et al. Immune and malignant cell phenotypes of ovarian cancer are determined by distinct mutational processes. *bioRxiv*. 2021.
18. González-Martín A, Pothuri B, Vergote I, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med*. 2019;381(25):2391-2402. doi:10.1056/nejmoa1910962
19. Millstein J, Budden T, Goode EL, et al. Prognostic gene expression signature for high-grade serous ovarian cancer. *Ann Oncol*. 2020;31:1240-1250.
20. Farley J, Smith LM, Darcy KM, et al. Cyclin E expression is a significant predictor of survival in advanced, suboptimally debulked ovarian epithelial cancers: a Gynecologic Oncology Group study. *Cancer Res*. 2003;63:1235-1241.
21. Wang X, Qi X, Ming X, Wang L, Wang Y, Zhao X. Prognostic value of cyclin E expression in patients with ovarian cancer: a meta-analysis. *J BUON*. 2017;22:64-71.
22. Köbel M, Kalløger SE, Boyd N, et al. Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. *PLoS Med*. 2008;5(12):e232. doi:10.1371/journal.pmed.0050232
23. Aziz D, Etemadmoghadam D, Caldon CE, et al. 19q12 amplified and non-amplified subsets of high grade serous ovarian cancer with overexpression of cyclin E1 differ in their molecular drivers and clinical outcomes. *Gynecol Oncol*. 2018;151(2):327-336. doi:10.1016/j.ygyno.2018.08.039
24. Sieh W, Köbel M, Longacre TA, et al. Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study. *Lancet Oncol*. 2013;14:853-862.
25. Köbel M, Kalløger SE, Lee S, et al. Biomarker-based ovarian carcinoma typing: a histologic investigation in the ovarian tumor tissue analysis consortium. *Cancer Epidemiol Biomark Prev*. 2013;22(10):1677-1686. doi:10.1158/1055-9965.epi-13-0391
26. Rambau PF, Vierkant RA, Intermaggio MP, et al. Association of p16 expression with prognosis varies across ovarian carcinoma histotypes: an Ovarian Tumor Tissue Analysis consortium study. *J Pathol Clin Res*. 2018;4:250-261. doi:10.1002/cjp2.109
27. Garsed DW, Alsop K, Fereday S, et al. Homologous recombination DNA repair pathway disruption and retinoblastoma protein loss are associated with exceptional survival in high-grade serous ovarian cancer. *Clin Cancer Res*. 2018;24(3):569-580. doi:10.1158/1078-0432.ccr-17-1621
28. Kang EY, Millstein J, Popovic G, et al. MCM3 is a novel proliferation marker associated with longer survival for patients with tubo-ovarian high-grade serous carcinoma. *Virchows Arch*. 2022;480:855-871.
29. Talhouk A, Kommos S, Mackenzie R, et al. Single-patient molecular testing with NanoString nCounter data using a reference-based strategy for batch effect correction. *PLoS One*. 2016;11(4):e0153844. doi:10.1371/journal.pone.0153844
30. Talhouk A, George J, Wang C, et al. Development and validation of the gene expression predictor of high-grade serous ovarian carcinoma molecular SubTYPE (PrOTYPE). *Clin Cancer Res*. 2020;26:5411-5423.
31. Köbel M, Piskorz AM, Lee S, et al. Optimized p53 immunohistochemistry is an accurate predictor of TP53 mutation in ovarian carcinoma. *J Pathol Clin Res*. 2016;2(4):247-258. doi:10.1002/cjp2.53
32. Lepage CC, Palmer MCL, Farrell AC, et al. Reduced SKP1 and CUL1 expression underlies increases in cyclin E1 and chromosome instability in cellular precursors of high-grade serous ovarian cancer. *Br J Cancer*. 2021;124(10):1699-1710. doi:10.1038/s41416-021-01317-w
33. Spruck C, Sun D, Fiegl H, et al. Detection of low molecular weight derivatives of cyclin E1 is a function of cyclin E1 protein levels in breast cancer. *Cancer Res*. 2006;66(14):7355-7360. doi:10.1158/0008-5472.can-05-3240
34. Theurillat JP, Metzler SC, Henzi N, et al. URI is an oncogene amplified in ovarian cancer cells and is required for their survival. *Cancer Cell*. 2011;19(3):317-332. doi:10.1016/j.ccr.2011.01.019
35. Lee JM, Minasian L, Kohn EC. New strategies in ovarian cancer treatment. *Cancer*. 2019;125(suppl 24):4623-4629. doi:10.1002/cncr.32544
36. Au-Yeung G, Lang F, Azar WJ, et al. Selective targeting of cyclin E1-amplified high-grade serous ovarian cancer by cyclin-dependent kinase 2 and AKT inhibition. *Clin Cancer Res*. 2017;23(7):1862-1874. doi:10.1158/1078-0432.ccr-16-0620
37. Geng Y, Michowski W, Chick JM, et al. Kinase-independent function of E-type cyclins in liver cancer. *Proc National Acad Sci USA*. 2018;115(5):1015-1020. doi:10.1073/pnas.1711477115
38. Lheureux S, Cristea MC, Bruce JP, et al. Adavosertib plus gemcitabine for platinum-resistant or platinum-refractory recurrent ovarian cancer: a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet*. 2021;397(10271):281-292. doi:10.1016/s0140-6736(20)32554-x
39. Au-Yeung G, Bressel M, Prall O, et al. A phase II signal-seeking trial of adavosertib targeting recurrent high-grade, serous ovarian cancer with cyclin E1 overexpression with and without gene amplification. *J Clin Oncol*. 2022;40(16_suppl):5515. doi:10.1200/jco.2022.40.16_suppl.5515

40. Gallo D, Young JTF, Fourtounis J, et al. CCNE1 amplification is synthetic lethal with PKMYT1 kinase inhibition. *Nature*. 2022;604(7907):749-756. doi:10.1038/s41586-022-04638-9

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kang E-Y, Weir A, Meagher NS, et al. CCNE1 and survival of patients with tubo-ovarian high-grade serous carcinoma: an Ovarian Tumor Tissue Analysis consortium study. *Cancer*. 2023;129(5):697-713. doi:[10.1002/cncr.34582](https://doi.org/10.1002/cncr.34582)