Volume 32, Issue 6

February 20, 2014

JOURNAL OF CLINICAL ONCOLOGY

Official Journal of the American Society of Clinical Oncology

ASCO 50 YEARS 1964-2014

FEB 28 2014
LIBRARY

Featured Content

Special Article

IMWG Consensus Statement for the Management, Treatment, and Supportive Care of Patients With Myeloma Not Eligible for Standard Autologous Stem-Cell Transplantation
A. Palumbo et al

Reducing Local Therapy in Patients Responding to Preoperative Systemic Therapy. *L.B. Marks et al*

Locoregional Radiotherapy in Patients With Breast Cancer Responding to Neoadjuvant Chemotherapy *J. White et al*

Identification of Potentially Avoidable Hospitalizations in Patients With GI Cancer. *G.A. Brooks et al*

Nomogram to Predict Cycle-One Serious Drug-Related Toxicity in Phase I Oncology Trials. *D.M. Hyman et al*
Editorial: *K.V. Ballman*

Ototoxicity in Children With High-Risk Neuroblastoma *W. Landier et al*

Concomitant Cisplatin Plus Radiotherapy and High-Dose-Rate Brachytherapy Versus Radiotherapy Alone for Stage IIIB Epidermoid Cervical Cancer *A.C. Zuliani et al*

Biology of Neoplasia: Liquid Biopsies: Genotyping Circulating Tumor DNA. *L.A. Diaz Jr et al*

ASCO

NeoGenomics
Exhibit 1092 Page 1

Volume 32, Issue 6 February 20, 2014

JOURNAL OF CLINICAL ONCOLOGY

Official Journal of the American Society of Clinical Oncology

CONTENTS

EDITORIALS

Timing Versus Duration of Adjuvant Therapy for Pancreatic Cancer: All the Lessons We Need in Life Are Taught to Us As Children
Emily Chan and Jordan Berlin (see article on page 504) 487

Phase I Trial Improvement: A Question of Patient Selection, Trial Design, or Both?
Karla V. Ballman (see article on page 519) 489

COMMENTS AND CONTROVERSIES

Reducing Local Therapy in Patients Responding to Preoperative Systemic Therapy: Are We Outsmarting Ourselves?
Lawrence B. Marks and Leonard R. Prosnitz (see article on page 494) 491

Locoregional Radiotherapy in Patients With Breast Cancer Responding to Neoadjuvant Chemotherapy: A Paradigm for Treatment Individualization
Julia White and Eleftherios Mamounas (see article on page 491) 494

ORIGINAL REPORTS

Rapid Communication

I RC **Identification of Potentially Avoidable Hospitalizations in Patients With GI Cancer**
Gabriel A. Brooks, Thomas A. Abrams, Jeffrey A. Meyerhardt, et al 496

continued on following page

Journal of Clinical Oncology (ISSN 0732-183X) is published 36 times a year, three times monthly, by the American Society of Clinical Oncology, 2318 Mill Road, Suite 800, Alexandria, VA 22314. Periodicals postage is paid at Alexandria, VA, and at additional mailing offices. Publication Mail Agreement Number 863289.

Editorial correspondence should be addressed to Stephen A. Cannistra, MD, *Journal of Clinical Oncology*, 2318 Mill Road, Suite 800, Alexandria, VA 22314. Phone: 703-797-1900; Fax: 703-684-8720. E-mail: jco@asco.org. Internet: www.jco.org.

Journal of Clinical Oncology® is a registered trademark of American Society of Clinical Oncology, Inc.

POSTMASTER: ASCO members should send changes of address to American Society of Clinical Oncology, 2318 Mill Road, Suite 800, Alexandria, VA 22314. Nonmembers should send changes of address to *Journal of Clinical Oncology* Customer Service, 2318 Mill Road, Suite 800, Alexandria, VA 22314.

2014 annual subscription rates, effective September 1, 2013: United States and possessions: individual, $607 one year, $1,153 two years; single issue, $40. International: individual, $842 one year, $1,600 two years; single issue, $50. Institutions: bundled (print + online): Tier 1: $944 US, $1,310 Int'l; Tier 2: $1,089 US, $1,444 Int'l; Tier 3: $1,572 US, $1,914 Int'l; Tier 4: contact *JCO* for quote. Institutions: online only, worldwide: Tier 1: $807; Tier 2: $924; Tier 3: $1,334; Tier 4: contact *JCO* for quote. See www.jco.org/ratecard for descriptions of each tier. Student and resident: United States and possessions: $289; all other countries, $401. To receive student/resident rate, orders must be accompanied by name of affiliated institution, date of term, and the signature of program/residency coordinator on institution letterhead. Orders will be billed at individual rate until proof of status is received. Current prices are in effect for back volumes and back issues. Back issues sold in conjunction with a subscription rate are on a prorated basis. Subscriptions are accepted on a 12-month basis. Prices are subject to change without notice. Single issues, both current and back, exist in limited quantities and are offered for sale subject to availability. *JCO* Legacy Archive (electronic back issues from January 1983 through December 1998) is also available; please inquire.

NeoGenomics
Exhibit 1092 Page 2

Volume 32, Issue 6 February 20, 2014

Gastrointestinal Cancer

Optimal Duration and Timing of Adjuvant Chemotherapy After Definitive Surgery for Ductal Adenocarcinoma of the Pancreas: Ongoing Lessons From the ESPAC-3 Study
Juan W. Valle, Daniel Palmer, Richard Jackson, et al (see editorial on page 487) 504

Neoadjuvant Chemotherapy Without Routine Use of Radiation Therapy for Patients With Locally Advanced Rectal Cancer: A Pilot Trial
Deborah Schrag, Martin R. Weiser, Karyn A. Goodman, et al 513

Clinical Trial

Nomogram to Predict Cycle-One Serious Drug-Related Toxicity in Phase I Oncology Trials
David M. Hyman, Anne A. Eaton, Mrinal M. Gounder, et al et al (see editorial on page 489) 519

Pediatric Oncology

Ototoxicity in Children With High-Risk Neuroblastoma: Prevalence, Risk Factors, and Concordance of Grading Scales—A Report From the Children's Oncology Group
Wendy Landier, Kristin Knight, F. Lennie Wong, et al 527

Neurooncology

I **Effect of the Addition of Chemotherapy to Radiotherapy on Cognitive Function in Patients With Low-Grade Glioma: Secondary Analysis of RTOG 98-02**
Roshan S. Prabhu, Minhee Won, Edward G. Shaw, et al 535

Gynecologic Cancer

I **Concomitant Cisplatin Plus Radiotherapy and High-Dose-Rate Brachytherapy Versus Radiotherapy Alone for Stage IIIB Epidermoid Cervical Cancer: A Randomized Controlled Trial**
Antonio Carlos Zuliani, Sergio Carlos Barros Esteves, Luiz Carlos Teixeira, et al 542

Hematologic Malignancy

I **Epigenetics Meets Genetics in Acute Myeloid Leukemia: Clinical Impact of a Novel Seven-Gene Score**
Guido Marcucci, Pearlly Yan, Kati Maharry, et al 548

Cancer Prevention and Control

I **Randomized Controlled Trial of a Cognitive-Behavioral Therapy Plus Hypnosis Intervention to Control Fatigue in Patients Undergoing Radiotherapy for Breast Cancer**
Guy H. Montgomery, Daniel David, Maria Kangas, et al 557

Thoracic Oncology

Second-Hand Smoke As a Predictor of Smoking Cessation Among Lung Cancer Survivors
Lawson Eng, Jie Su, Xin Qiu, et al 564

Urologic Oncology

Longitudinal Serum Testosterone, Luteinizing Hormone, and Follicle-Stimulating Hormone Levels in a Population-Based Sample of Long-Term Testicular Cancer Survivors
Mette Sprauten, Marianne Brydøy, Hege S. Haugnes, et al 571

continued on following page

NeoGenomics
Exhibit 1092 Page 3

Volume 32, Issue 6 February 20, 2014

BIOLOGY OF NEOPLASIA

Liquid Biopsies: Genotyping Circulating Tumor DNA
Luis A. Diaz Jr and Alberto Bardelli 579

SPECIAL ARTICLE

International Myeloma Working Group Consensus Statement for the Management, Treatment, and Supportive Care of Patients With Myeloma Not Eligible for Standard Autologous Stem-Cell Transplantation
Antonio Palumbo, S. Vincent Rajkumar, Jesus F. San Miguel, et al 587

ART OF ONCOLOGY

The Perfect Arrangement
Sarah P. Blagden 601

CORRESPONDENCE

Public Database of Federally Funded Clinical Trials Data
William Read 603

Reply to W. Read
Agnes Dechartres and Philippe Ravaud 603

Reply to W. Read
Sandip M. Prasad, Oliver Sartor, and Charles L. Bennett 604

Are Dose-Dense and Triplet Chemotherapy Regimens Optimal Adjuvant Therapy in the Majority of Women With Node-Positive Early Breast Cancer?
Eugene J. Moylan, Louise C. Connell, and Seamus O'Reilly 605

Reply to E.J. Moylan et al
Sandra M. Swain, Gong Tang, and Charles E. Geyer Jr 606

Guidelines for Long-Term Steroid Therapy in End-of-Life Palliative Care
Hiromichi Yamane, Nobuaki Ochi, Tomoko Yamagishi, et al 607

Dexamethasone for Cancer-Related Fatigue
Michael Franco, Leeroy William, Peter Poon, et al 608

Reply to H. Yamane et al and M. Franco et al
Sriram Yennurajalingam and Eduardo Bruera 609

continued on following page

Volume 32, Issue 6 February 20, 2014

DIAGNOSIS IN ONCOLOGY

(articles available online)

Diagnostic and Treatment Dilemma of Dual Pathology of Lung Cancer and Disseminated Tuberculosis
Nishitha Shetty, Vanita Noronha, Amit Joshi, et al e7

Renal Carcinoma Associated With Succinate Dehydrogenase B Mutation: A New and Unique Subtype of Renal Carcinoma
Julie Y. Paik, Christopher W. Toon, Diana E. Benn, et al e10

Also in This Issue

Information for Subscribers
Information for Contributors
Manuscript Submission Checklist
Current Abstracts
Forthcoming Reports

RC Rapid Communication: published within 4 to 6 weeks after acceptance, with free access

I Supplementary Information Available

www.jco.org

NeoGenomics
Exhibit 1092 Page 5

JOURNAL OF CLINICAL ONCOLOGY
Official Journal of the American Society of Clinical Oncology

EDITOR ROSTER

EDITOR-IN-CHIEF
Stephen A. Cannistra, MD, *Boston, MA*

DEPUTY EDITOR:
TRANSLATIONAL ONCOLOGY
Mary L. Disis, MD, *Seattle, WA*

DEPUTY EDITOR:
INTERNATIONAL EDITIONS
David M. Khayat, MD, PhD, *Paris, France*

ASSOCIATE EDITORS
Judith Abrams, PhD, *Detroit, MI*
Karla V. Ballman, PhD, *Rochester, MN*
Michael A. Carducci, MD, FACP, *Baltimore, MD*
Jonathan W. Friedberg, MD, *Rochester, NY*
Pamela J. Goodwin, MD, *Toronto, Canada*
Bruce G. Haffty, MD, *New Brunswick, NJ*
Melissa M. Hudson, MD, *Memphis, TN*
Paul B. Jacobsen, PhD, *Tampa, FL*
Stephanie R. Land, PhD, *Bethesda, MD*
T. Andrew Lister, MD, *London, United Kingdom*
Danny Rischin, MD, *Melbourne, Australia*
Joan H. Schiller, MD, *Dallas, TX*
Gary K. Schwartz, MD, *New York, NY*
Joel E. Tepper, MD, *Chapel Hill, NC*
Alan P. Venook, MD, *San Francisco, CA*
Jaap Verweij, MD, PhD, *Rotterdam, the Netherlands*
Antonio C. Wolff, MD, *Baltimore, MD*

CONSULTANT EDITORS
Harold J. Burstein, MD, PhD—*Medical Education*
Levi A. Garraway, MD, PhD—*Translational Oncology*
Mark N. Levine, MD—*Health Research Methodology*
Lidia Schapira, MD—*Art of Oncology*

EDITORS-IN-CHIEF EMERITI
Joseph R. Bertino, MD, *New Brunswick, NJ*
George P. Canellos, MD, *Boston, MA*
Daniel G. Haller, MD, *Philadelphia, PA*

INTERNATIONAL EDITORS
Brazil Edition
Rogerio Lilenbaum, MD, *New Haven, CT*
Antonio Carlos Buzaid, MD, *São Paolo, Brazil*

Czech/Slovak Edition
Jindrich Finek, MD, PhD,
Plzen, Czech Republic

French Edition
Jean-Philippe Spano, MD, PhD,
Paris, France

Japanese Edition
Nagahiro Saijo, MD, PhD, *Tokyo, Japan*

Middle East Edition
Nagi S. El-Saghir, MD, *Beirut, Lebanon*

North African Edition
Nagi S. El-Saghir, MD, *Beirut, Lebanon*

Russian Edition
Irina Vladimirovna Poddubnaya, MD, PhD,
Moscow, Russia

South African Edition
Raymond P. Abratt, MD, *Cape Town, South Africa*

Spanish Edition
Josep Tabernero, MD, *Barcelona, Spain*

PUBLISHER
David Sampson

MANAGING EDITORS
Ken G. Kornfield
Glenn Landis
Emilie Gunn

CUSTOMER SERVICE
Phone: 703-519-1430
Fax: 703-518-8155
E-mail: jcoservice@asco.org

EDITORIAL OFFICE
2318 Mill Road, Suite 800
Alexandria, VA 22314
Phone: 703-797-1900
Fax: 703-684-8720
E-mail: jco@asco.org

ADVERTISING SALES
The Walchli Tauber Group, Inc.
225 Old Emmorton Road, Suite 201
Bel Air, MD 21015
Phone: 443-512-8899
Fax: 443-512-8909
Internet: www.wt-group.com

BUSINESS-TO-BUSINESS SALES
Rick Werdann
Springer Healthcare, LLC
233 Spring Street
New York, NY 10013
Phone: 212-460-1523
Mobile: 646-209-1840
E-mail: rick.werdann@springer.com
Internet: www.SpringerHealthcare.com

JOURNAL OF CLINICAL ONCOLOGY

Official Journal of the American Society of Clinical Oncology

Journal of Clinical Oncology (ISSN 0732-183X) is published 36 times a year, three times monthly, by the American Society of Clinical Oncology, 2318 Mill Road, Suite 800, Alexandria, VA 22314. Periodicals postage is paid at Alexandria, VA, and at additional mailing offices.

Postmaster

Send all changes of address for *Journal of Clinical Oncology* subscribers to:
JCO Customer Service
2318 Mill Road, Suite 800
Alexandria, VA 22314

Editorial Correspondence (manuscript-related inquiries):
Stephen A. Cannistra, MD, Editor-in-Chief
Journal of Clinical Oncology
2318 Mill Road, Suite 800
Alexandria, VA 22314
Phone: 703-797-1900; Fax: 703-684-8720
E-mail: jco@asco.org; Internet: www.jco.org

American Society of Clinical Oncology (membership-related inquiries):
ASCO Member Services
2318 Mill Road, Suite 800
Alexandria, VA 22314
Phone: 703-299-0158; Toll-free: 888-282-2552
Fax: 703-299-0255
E-mail: membermail@asco.org; Internet: www.asco.org
Hours: Monday-Friday, 8:30 a.m.-5:00 p.m. Eastern Time

Customer Service, Subscriptions, and Changes of Address:
JCO Customer Service
2318 Mill Road, Suite 800
Alexandria, VA 22314
Phone: 703-519-1430; Toll-free: 888-273-3508; Fax: 703-518-8155
E-mail: jcoservice@asco.org
Internet orders/renewals: www.jco.org/subscriptions

2014 SUBSCRIPTION RATES

Individual Prices

Domestic (US) Print + Online
Individuals in training $303
Individuals (1 year) $607

International Print + Online
Individuals in training $421
Individuals (1 year) $842

Institutional Prices

Domestic (US) Print + Online	Online Only
Tier 1 $944	$807
Tier 2 $1,089	$924
Tier 3 $1,572	$1,334
Tier 4 Call for quote	Call for quote

International Print + Online	Online Only
Tier 1 $1,310	$807
Tier 2 $1,444	$924
Tier 3 $1,914	$1,334
Tier 4 Call for quote	Call for quote

Orders and Payments
P.O. Box 37211
Baltimore, MD 21279-3211

Important Tiers and Pricing Notes
Additional rates along with tier descriptions are available online at www.jco.org/ratecard

1. Prices are in effect from September 1, 2013, through August 31, 2014. Prices are subject to change.
2. Print-only subscriptions or additional print subscriptions are available for $795 in the US and $1,150 outside the US.
3. Institutional online access, whether an online-only or bundled subscription, is for a single-site license, which allows an unlimited number of concurrent users from that site.
4. For multisite licenses, please contact the appropriate agent for a quote.
5. Subscribers outside the US, add $100 per print subscription for expedited delivery.
6. Single-issue price: $40 US, $50 international.
7. Prices quoted are in US dollars and payments must be made in US dollars.
8. Except on Tier 5 orders, the publisher allows for a 5% discount to recognized subscription agents.

Prices are subject to change without notice. Current prices are in effect for back volumes and back issues. Single issues, both current and back, exist in limited quantities and are offered for sale subject to availability. Back issues sold in conjunction with a subscription are on a prorated basis.

Advertising Sales
The Walchli Tauber Group, Inc.
225 Old Emmorton Road, Suite 201
Bel Air, MD 21015
Phone: 443-512-8899; Fax: 443-512-8909
Internet: www.wt-group.com

Business-to-Business Sales
Rick Werdann
Springer Healthcare, LLC
233 Spring Street
New York, NY 10013
Phone: 212-460-1523; Mobile: 646-209-1840
E-mail: rick.werdann@springer.com
Internet: www.SpringerHealthcare.com

LICENSES AND CONSORTIA

USA, Canada, and Europe
David Charles
eLicensing
92 Avenue du General de Gaulle
78600 Maisons-Laffitte, France
Phone/Fax: +33-1-39-12-29-29
E-mail: dc.elicensing@orange.fr

Japan
USACO Corporation
2-17-12 Higashi-Azuba Minato-ku
Tokyo, Japan 106-0044
Phone: +81-3-3505-3529; Fax: +81-3-3505-6284
E-mail: import@usaco.co.jp; Internet: www.usaco.co.jp

JOURNAL OF CLINICAL ONCOLOGY
Official Journal of the American Society of Clinical Oncology

China
Charlesworth China
Beijing Modern Palace Building, 12th Floor
No. 20, Dongsanhuan Nanlu
Chaoyang District
Beijing 100022
PR China
Phone: +86-10-6779-1601; Fax: +86-10-6779-9806
E-mail: sales@charlesworth.com.cn
Internet: www.charlesworth.com.cn (in Mandarin)
and www.charlesworth.com

India
Publishers Communication Group
F-110, Jawahar Park, Khanpur
New Delhi-110062
India
Phone: +91-9891096706
E-mail: dkumar@pcgplus.com

Indonesia, Malaysia, Philippines, Singapore, South Korea, Thailand, Taiwan, and Vietnam
EBSCO EMpact
5724 Highway 80 East
Birmingham, AL 35242
Phone: +1-205-980-6676
Fax: +852-2575-8822
E-mail: jmcdaniel@ebsco.com

Central/South America, The Caribbean
Accucoms (US), Inc.
West Point Commons
1816 West Point Pike, Suite 201
Lansdale, PA 19446
Phone: 215-395-5026
Fax: 215-660-5042
E-mail: anouk.snijders@accucoms.com
Internet: www.accucoms.com

Permissions Requests
Licensing, Rights, and Permissions Division
American Society of Clinical Oncology
2318 Mill Road, Suite 800
Alexandria, VA 22314
Phone: 571-483-1722; Fax: 703-518-5094
E-mail: permissions@asco.org

Free Public Access
Journal of Clinical Oncology (*JCO*) provides free online access to original research articles older than one year at www.jco.org. In addition, all ASCO Special Articles, Rapid Communications, Editorials, Comments and Controversies articles, the Art of Oncology series, and Correspondence articles are free immediately upon publication.

Disclaimer
The ideas and opinions expressed in *JCO* do not necessarily reflect those of the American Society of Clinical Oncology (ASCO). The mention of any product, service, or therapy in this publication or in any advertisement in this publication should not be construed as an endorsement of the products mentioned. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge of the patient, to determine drug dosages and the best treatment for the patient. Readers are advised to check the appropriate medical literature and the product information currently provided by the manufacturer of each drug to be administered to verify approved uses, the dosage, method, and duration of administration, or contraindications. Readers are also encouraged to contact the manufacturer with questions about the features or limitations of any products. ASCO assumes no responsibility for any injury or damage to persons or property arising out of or related to any use of the material contained in this publication or to any errors or omissions.

Copyright
Copyright © 2014 by American Society of Clinical Oncology unless otherwise indicated. All rights reserved. No part of this publication may be reproduced or transmitted in any form or by any means now or hereafter known, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the Publisher. Printed in the United States of America.

The appearance of the code at the bottom of the left column of the first page of an article in this journal indicates the copyright owner's consent that copies of the article may be made for personal or internal use, or for the personal or internal use of specific clients, for those registered with the Copyright Clearance Center, Inc. (222 Rosewood Drive, Danvers, MA 01923; 978-750-8400; www.copyright.com). This consent is given on the condition that the copier pay the stated per-copy fee for that article through the Copyright Clearance Center, Inc., for copying beyond that permitted by Sections 107 or 108 of the US Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale. Absence of the code indicates that the material may not be processed through the Copyright Clearance Center, Inc.

CPT © is a trademark of the American Medical Association.

Journal of Clinical Oncology® is a registered trademark of American Society of Clinical Oncology, Inc.

VOLUME 32 · NUMBER 6 · FEBRUARY 20 2014

JOURNAL OF CLINICAL ONCOLOGY

BIOLOGY OF NEOPLASIA

Liquid Biopsies: Genotyping Circulating Tumor DNA

Luis A. Diaz Jr and Alberto Bardelli

Luis A. Diaz Jr, Swim Across America Laboratory and Ludwig Center for Cancer Genetics and Therapeutics, Johns Hopkins University School of Medicine, Baltimore, MD; and Alberto Bardelli, Institute for Cancer Research and Treatment at Candiolo, University of Torino, Candiolo, and the Fondazione Italiana per la Ricerca sul Cancro Institute of Molecular Oncology, Milan, Italy.

Published online ahead of print at www.jco.org on January 21, 2014.

Supported by the European Union Seventh Framework Programme of the Colon Therapy Research Consortium (Grant No. 259015); by the Associazione Italiana per la Ricerca sul Cancro (AIRC) Investigator Grant No. 12812; by AIRC 2010 Special Program Molecular Clinical Oncology 5 per mille, Project No. 9970; by Fondazione Piemontese per la Ricerca sul Cancro 5 per mille 2010 Ministero della Salute; Ministero dell'Istruzione, dell'Università e della Ricerca (Progetti di Ricerca di Interesse Nazionale), and by Swim Across America.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Alberto Bardelli, PhD, Laboratory of Molecular Genetics, Institute for Cancer Research and Treatment, Department of Oncology, University of Torino, Medical School, Str prov 142 Km 3.95, 10060 Candiolo, Torino, Italy; e-mail: a.bardelli@unito.it.

© 2014 by American Society of Clinical Oncology

0732-183X/14/3206-579/$20.00

DOI: 10.1200/JCO.2012.45.2011

ABSTRACT

Genotyping tumor tissue in search of somatic genetic alterations for actionable information has become routine practice in clinical oncology. Although these sequence alterations are highly informative, sampling tumor tissue has significant inherent limitations; tumor tissue is a single snapshot in time, is subject to selection bias resulting from tumor heterogeneity, and can be difficult to obtain. Cell-free fragments of DNA are shed into the bloodstream by cells undergoing apoptosis or necrosis, and the load of circulating cell-free DNA (cfDNA) correlates with tumor staging and prognosis. Moreover, recent advances in the sensitivity and accuracy of DNA analysis have allowed for genotyping of cfDNA for somatic genomic alterations found in tumors. The ability to detect and quantify tumor mutations has proven effective in tracking tumor dynamics in real time as well as serving as a liquid biopsy that can be used for a variety of clinical and investigational applications not previously possible.

J Clin Oncol 32:579-586. © 2014 by American Society of Clinical Oncology

INTRODUCTION

Fragmented DNA is found in circulation in the cell-free component of whole blood. Initially reported by Mandel and Metais[1] in 1948, the clinical utility of circulating cell-free DNA (cfDNA) in the serum and plasma has been an area of active research in many disciplines of medicine. Evaluation of fetal DNA in the circulation of expecting mothers has seen the most success.[2-4] Investigation of fetal DNA can now uncover germline fetal changes weeks after conception, including point mutation and aneuploidy, and is likely to become part of the standard of care in prenatal assessment in high-risk patients.[5,6]

Investigation of cfDNA has included other clinical scenarios such as exercise, end-stage renal failure, stroke, myocardial infarction, surgery, and trauma.[7-20] These studies have demonstrated that circulating cfDNA exists at steady-state levels and increases, sometimes dramatically, with cellular injury or necrosis.[17]

In oncology, detection of cfDNA derived from tumors, also known as circulating tumor DNA (ctDNA), has been challenging for three primary reasons, which include: discrimination of ctDNA from normal cfDNA; presence of sometimes extremely low levels of ctDNA; and the accurate quantification of the number of mutant fragments in a sample.

Discriminating ctDNA from normal cfDNA is aided by the fact that tumor DNA is defined by the presence of mutations. These somatic mutations, commonly single base-pair substitutions, are present only in the genomes of cancer cells or precancerous cells and are not present in the DNA of normal cells of the same individual. This juxtaposition assures ctDNA exquisite biologic specificity as a biomarker. Accordingly, all DNA sequencing methodologies that identify somatic variants could be used easily to identify ctDNA if tumor DNA fragments were abundant in the circulation of patients with cancer. Unfortunately, detection of cfDNA derived from tumors carries substantial challenges, largely because ctDNA often represents a small fraction (< 1.0%) of total cfDNA.[17,21,22] Therefore, standard sequencing approaches like Sanger sequencing or pyrosequencing can only detect tumor-derived mutant fragments in patients with heavy tumor burden and high levels of ctDNA.

Investigation of cfDNA in patients with cancer has recently increased, largely because of digital genomic technologies that allow for enumeration of rare mutant variants in complex mixtures of DNA. Before the introduction of techniques like digital polymerase chain reaction (PCR),[23] beads, emulsion, amplification, and magnetics (BEAMing),[24] or pyrophosphorolysis-activated polymerization (PAP),[25] detection of cfDNA derived from tumors was inconsistently detected,[26-29] with several reports suggesting that ctDNA measurement was inferior to that of other biomarkers, such as circulating tumor cells[30-32] (Fig 1). In advanced tumors, digital genomic approaches have high sensitivity, with the mutation identified in the tumor tissue matching the mutation in the ctDNA fraction in virtually every

This material may be protected by Copyright law (Title 17 U.S. Code)

NeoGenomics
Exhibit 1092 Page 9

Technique	Sensitivity	Optimal Application
Sanger sequencing	> 10%	Tumor tissue
Pyrosequencing	10%	Tumor tissue
Next-generation seqeuncing	2%	Tumor tissue
Quantative PCR	1%	Tumor tissue
ARMS	0.10%	Tumor tissue
BEAMing, PAP, Digital PCR, TAM-Seq	0.01% or lower	ctDNA, rare variants in tumor tissue

Fig 1. Methodologies for detecting circulating tumor DNA (ctDNA). Sanger sequencing (dideoxy-terminator sequencing),[33] amplification refractory mutation system (ARMS),[34,35] pyrosequencing,[36] pyrophosphorolysis-activated polymerization (PAP),[25] tagged-amplicon deep sequencing (TAM-Seq),[37] digital polymerase chain reaction (PCR),[23] and beads, emulsion, amplification, and magnetics (BEAMing).[38]

case.[17,21,39] Recently, PCR-based digital approaches have been updated with techniques that use next-generation sequencing (NGS) to identify rare mutant variants in complex mixtures of DNA (Table 1).[37,40-42] These techniques have expanded the ability to detect a single point mutation, and now multiple genes of interest can be investigated in one sample. Amplifications, rearrangements, and aneuploidy may now be detectable as well[17,41,43-45] (Fig 1).

The ability to detect and enumerate ctDNA creates a wide array of practical clinical applications that are not possible with routine sequencing of tumor tissue or with other circulating biomarkers (Table 1). This review will highlight some of these applications, in addition to the biology of ctDNA, and discuss promising future applications to solve unmet clinical needs.

MECHANISMS OF TUMOR DNA SHEDDING

In general, patients with cancer have much higher levels of normal circulating cfDNA than healthy individuals.[46-54] As the tumor increases in volume, so too does the cellular turnover and hence the number of apoptotic and necrotic cells.[55,56] Under normal physiologic circumstances, apoptotic and necrotic remains are cleared by infiltrating phagocytes. This does not happen efficiently within the tumoral mass, leading to the accumulation of cellular debris and its inevitable release into the circulation (Fig 2).

When the length of cfDNA strands are measured, they often assume the classic ladder pattern in integer multiples of 180 base pairs,[57] characteristic of the apoptotic process.[55,58,59] In fact, most cfDNA fragments measure between 180 and 200 base pairs, suggesting that apoptosis likely produces the majority of cfDNA in circulation.[6,17,21,55] The passive release of cfDNA into the bloodstream from apoptotic or necrotic cells is dependent on the location, size, and vascularity of the tumor, perhaps accounting for the variability in cfDNA levels often observed.

Salient to this review, it is evident that a proportion of fragmented DNA in circulation is derived directly from the tumor. Multiple methods have shown that the fraction of circulating DNA contributed from the tumor varies greatly, between 0.01% and more than 90%.[17] As

Table 1. Applications of Liquid Biopsy

Application
Early detection
Assessment of molecular heterogeneity of overall disease
Monitoring of tumor dynamics
Identification of genetic determinants for targeted therapy
Evaluation of early treatment response
Monitoring of minimal residual disease
Assessment of evolution of resistance in real time

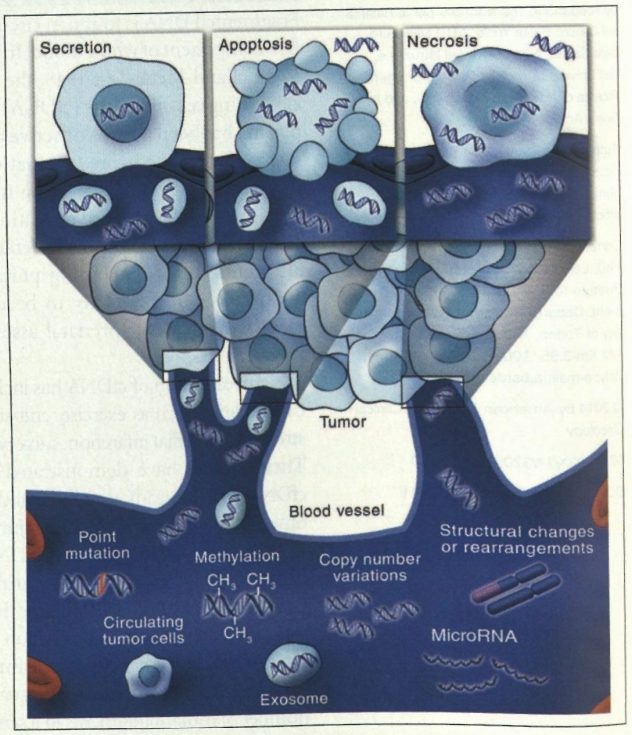


Fig 2. Genetic alterations detectable in circulating cell-free tumor DNA. Tumor cells release small fragments of cell-free DNA into circulation by multiple mechanisms. Cancer-associated genetic alterations such as point mutations, copy number variations, chromosomal rearrangements, and methylation patterns can be detected in circulating cell-free DNA.

 © 2014 by American Society of Clinical Oncology

NeoGenomics
Exhibit 1092 Page 10

discussed later in this review, the amount of ctDNA is related to the tumor burden and is therefore expected to vary significantly among individuals with different clinical histories.[17,43]

TISSUE VERSUS LIQUID BIOPSY

Many of the major recent advances in targeted therapies have relied on the acquisition of tumor tissue via biopsy before initiation of therapy or after the onset of resistance. The availability of tissue for molecular analysis has been instrumental in understanding the primary mechanism of action of agents such as trastuzumab, imatinib, cetuximab, and vemurafenib. Likewise, access to tumor tissue after clinical resistance has helped to define mechanisms of secondary resistance to these targeted agents, often in the very pathway or gene involved in their responsiveness.[60]

Although tumor tissue is the gold standard for clinical and investigational sequencing, major barriers exist in terms of acquisition and utility. Biopsies are an inconvenience from a scheduling perspective; they also increase the cost of patient care and are yet another uncomfortable, invasive procedure for patients that often do not influence outcome. Finally, and most importantly, biopsies are not without clinical complications. A review of the investigative biopsies at MD Anderson Cancer Center reported adverse event rates of 17.1% and 1.6% for thoracic biopsies and abdominal/pelvic sampling, respectively.[61]

In addition to the issues related to tissue acquisition, sample preservation and tumor heterogeneity also hamper the use of tumor tissue for cancer sequencing.[22] Most tumor tissue is preserved in formalin-fixed paraffin-embedded (FFPE) blocks, which crosslink DNA and in some cases can result in FFPE samples being inadequate for molecular analysis. Although this has improved for individual gene mutations and targeted gene sets, there are still limitations for whole genome and exome analyses. Furthermore, the quantity of tumor cells in each biopsy varies and is largely dependent on the tumor cellularity (percent tumor) and size of the specimen acquired. This is further compounded by small tissue amounts from fine-needle aspirates or core-needle biopsies, which often result in smaller amounts of tumor tissue for molecular analysis in comparison with surgically resected specimens.

Arguably, the major limitation of tissue biopsy is heterogeneity, which characterizes most advanced cancers.[62,63] Cancers are heterogeneous, with different areas of the same tumor showing different genetic profiles (ie, intratumoral heterogeneity); likewise, heterogeneity exists between metastases within the same patient (ie, intermetastatic heterogeneity). A biopsy or tissue section from one part of a solitary tumor will miss the molecular intratumoral as well as intermetastatic heterogeneity.

To overcome the limitations of tissue biopsies, less invasive techniques capable of capturing tumor heterogeneity and the molecular changes cancer cells undergo when they are exposed to therapy are needed.[44,64,65] Circulating tumor DNA can in principle provide the same genetic information as a tissue biopsy necessary to interrogate key companion diagnostics. Accessing the bloodstream has clear advantages. For one, it is a source of fresh DNA, unhampered by preservatives. Sampling the blood from a needle stick is minimally invasive and avoids the dangers of biopsies. Furthermore, blood can be drawn at any time during the course of therapy and allow for dynamic monitoring of molecular changes in the tumor rather than relying on a static time point.

What information can be derived from ctDNA? Circulating tumor DNA fragments contain genetic defects identical to those of the tumors themselves; these DNA alterations span the types of genomic alterations identified in the tumor and include point mutations (*EGFR* and *KRAS*), rearrangements (*EML4-ALK*), amplifications (*HER2* and *MET*), and even aneuploidy (Fig 2). Moreover, investigating plasma from patients can account for molecular heterogeneity, because ctDNA fragments are collected from all tumors in a patient's body through circulation (Fig 2).[44,65] Accordingly, examination of ctDNA for genetic alterations present in the tumor tissue is, in reality, a liquid biopsy. Although current evidence suggests that ctDNA likely represents a molecular proxy of the overall disease, it remains to be formally proven that multiple metastatic lesions located in different organs shed ctDNA homogeneously.

Although much of the current data for ctDNA are investigational, the sensitivity of these liquid biopsies for patients with stage IV disease seems to be approaching 100%.[17,21,39] Despite the promising results in advanced disease, the sensitivity of measuring ctDNA is reliant on biologic and technical factors. The abundance of tumor cells represented by tumor stage or overall tumor burden can dictate sensitivity. Lower-stage tumors and even advanced cases involving low-level micrometastatic disease have lower numbers of ctDNA fragments.[21] Technically, sensitivity is limited by the error rate of DNA polymerase, which is generally considered to be 0.01%.[38] Therefore, if the fraction of ctDNA in a sample is at or below 0.01%, it is considered negative for ctDNA.[17,38] Newer approaches aimed at increasing overall sensitivity using barcoding strategies in the context of NGS are being investigated.[40] This will be important because false-negative results, especially when ctDNA results are used for therapeutic decision making or in detecting occult disease, could be detrimental. The absolute sensitivity for ctDNA analysis in a variety of clinical scenarios will have to be evaluated to best understand the limitations and advantages of this new technology.

MONITORING TUMOR BURDEN

Liquid biopsies may also be useful in monitoring tumor burden, a central aspect in the management of patients with cancer that is typically assessed with imaging. Circulating biomarkers have played an increasingly important role in assessing disease burden, especially in circumstances where imaging delivers indeterminate results. PSA, cancer antigen (CA) 19-9, carcinoembryonic antigen, and CA-125 are examples of protein biomarkers that aid in assessment of therapeutic response. Unfortunately, many malignancies do not have a reliable protein biomarker, and even in those diseases with useful biomarkers, these markers often lack specificity and may be elevated as a result of clinical situations not related to tumor growth or progression. Furthermore, most protein biomarkers persist in circulation for weeks, thereby only allowing accurate assessment over weeks to months.[37,66-68]

There are clear advantages to measuring ctDNA as a marker of tumor dynamics over conventional protein biomarkers or even imaging studies. For one, ctDNA has a comparatively short half-life (approximately 2 hours), allowing for evaluation of tumor changes in hours rather than weeks to months.[17] Changes in ctDNA can predate those seen in imaging studies or using protein biomarkers by weeks to

© 2014 by American Society of Clinical Oncology

NeoGenomics
Exhibit 1092 Page 11

months.[17,43] Furthermore, ctDNA is exquisitely specific for an individual's tumor, because by definition somatic cancer mutations are identified by their presence in tumor DNA and absence in matched normal DNA. This bypasses the issues related to confusing false-positive results often encountered with other circulating biomarkers and imaging studies.

Several investigational studies have shown that ctDNA can be a surrogate for tumor burden and that much like viral load changes (eg, HIV viral load), levels of ctDNA correspond with clinical course. Studies in melanoma, ovarian, breast, and colon cancers have solidified the potential utility of this approach to more precisely define tumor dynamics during therapy for patients with advanced disease.[17,28,37,43,69] There are rapid increases in ctDNA levels with disease progression and corresponding declines in levels after successful treatment with pharmacologic therapy or resective surgery.[17,28,37,43]

Two potential processes have been described for performing ctDNA analysis. The first requires identifying a specific mutation or mutations in the tumor tissue to target and quantify the level of ctDNA at a particular time point.[17] The alternative approach is scanning regions of DNA extracted from plasma or serum for mutations of interest in a blinded manner because the tumor tissue was not initially assessed.[37,40,43] In both cases, the genotype is identified, and the mutation is quantified and represented in a number of mutant fragments per mL or as a mutant fraction (%) in an individual sample.

Clinical applications for this technology include monitoring tumor response to therapy and potentially defining ambiguous clinical scenarios like stable disease or mixed responses.[37] Furthermore, changes in ctDNA may predict for treatment response early in the course of therapy, which may allow for real-time modification of the treatment regimen, rather than waiting weeks or months to monitor response to therapy. This question is currently under investigation. All of these potential indications will have to be evaluated in the context of clinical trials also testing the benefit of novel therapeutic agents.

MINIMAL RESIDUAL DISEASE

Another potential application of ctDNA is the detection of minimal residual disease after surgery or therapy with curative intent. In these scenarios, as is the case in breast or colon cancer, resective surgery alone cures a large fraction of patients with localized disease. However, we have no effective means presently to identify which patients are cured and which have residual disease that will result in disease recurrence. Furthermore, those groups of patients with high-risk clinical and pathologic criteria are indiscriminately treated with adjuvant chemotherapy despite the fact that a large portion are cured and do not need such potentially toxic therapy.

Currently, predicting which patients are disease free after surgery (ie, cured) and those who have residual disease depends largely on clinical and pathologic parameters. The most important criterion has been the TNM staging system. This system stratifies patients by risk for recurrence and possible benefit from adjuvant chemotherapy. Recently, novel molecular features such as gene expression profiles have been used to improve the predictive power of the TNM staging system.[70] Assessed in the resected tumor specimen, these markers are biologic surrogates for phenotypes such as tumor age and aggressive biology. However, they do not address whether residual tumor is present after surgical resection.

Circulating tumor DNA is a potential marker of residual disease after resection and may determine which patients will experience recurrence. Optimally, ctDNA should be measured after surgery but before initiation of adjuvant therapy (in general, 6 to 8 weeks after surgery) to best facilitate therapeutic decision making.

The best example to date has been in a cohort of patients with colorectal cancer undergoing resection with curative intent.[17] A mutation profile was determined from each patient's resected tumor, and this personal and unique molecular signature was then used to create a set of mutation-specific probes for each patient. These probes were used to detect and quantify ctDNA after surgery in these patients undergoing potentially curative resection, and the patients were subsequently observed over the course of 2 to 5 years. In this study, ctDNA was shown to be sufficiently sensitive to detect minimal residual disease after surgical resection.[17] All patients with detectable postoperative levels of ctDNA experienced recurrence, whereas all patients with undetectable postoperative levels of ctDNA remained disease free. Similar studies have been performed blindly, examining *KRAS* mutations in patients with resected colorectal cancer, and shown a strong correlation between postoperative detection of mutant DNA in circulation and recurrence.[58]

Future studies evaluating postoperative levels of ctDNA could offer personalized markers based on the unique mutational profiles of resected tumors and thus having exquisitely high specificity. Sensitivity will rely on the ability to detect low levels of ctDNA released from micrometastatic deposits not detectable by imaging or other diagnostic modalities. Applications of such an approach have the potential to affect a broad array of tumors treated with curative intent.

MONITORING OF MOLECULAR RESISTANCE AND HETEROGENEITY

The emergence of clinical resistance to a previously effective antineoplastic therapy results from the acquisition of molecular alterations in genes or pathways that govern the mechanisms of action of the newly ineffective therapy. Defining these mechanisms of resistance to a targeted agent is often done using preclinical models (cell lines or xenografts in mice), because it is generally more difficult to identify and confirm these findings in clinical samples. In principle, every patient enrolled onto a therapeutic clinical trial should undergo a tissue biopsy before initiation of the experimental therapy and after progression. Molecular tools, such as NGS, can be implemented to find genetic differences between the tissue collected before and after therapy. This will offer a snapshot of the predominant resistant clone of a portion of the lesion under examination.

Recent studies have shown that liquid biopsies can be used effectively to monitor the emergence of multiple resistance clones during the course of treatment (Fig 3). The genetic bases of secondary resistance to various targeted drugs have been elucidated in great detail.[71,72] The most extensive analyses are those involving patients with Philadelphia chromosome–positive chronic myeloid leukemia treated with the tyrosine kinase inhibitor imatinib. Emergence of ABL kinase domain mutations have been extensively implicated in the pathogenesis of acquired resistance to imatinib.[73]

 © 2014 by American Society of Clinical Oncology

NeoGenomics
Exhibit 1092 Page 12

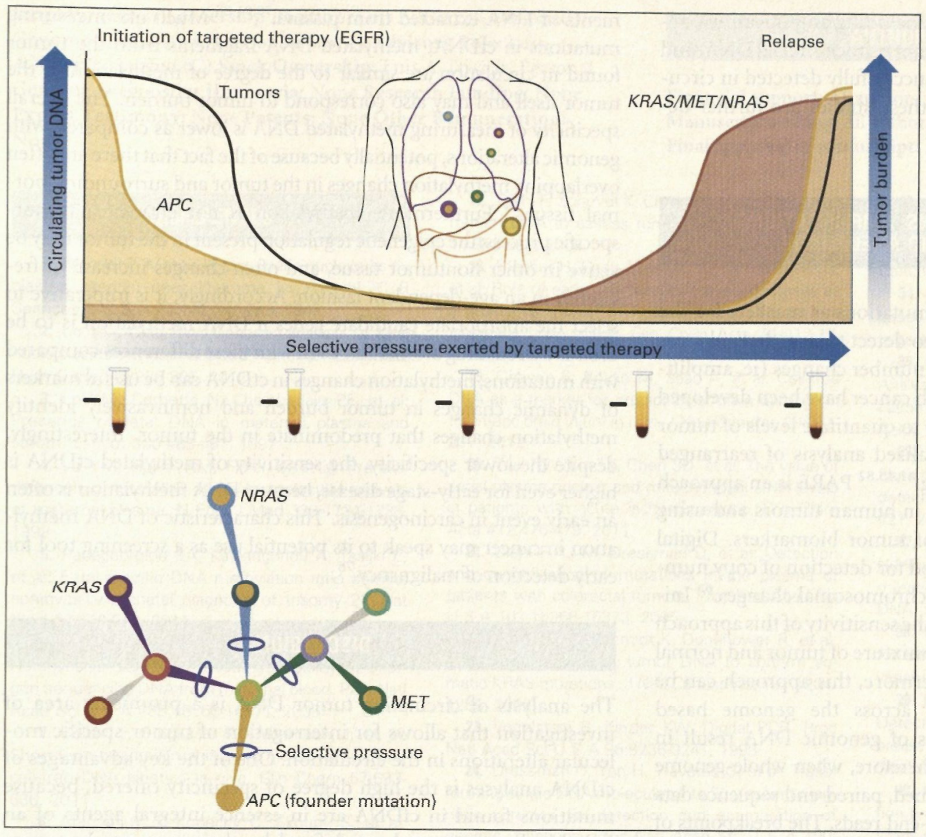


Fig 3. Detection of tumor-specific DNA mutations in the blood of patients to monitor response and relapse with targeted therapies. This schematic depicts a representation of a patient with metastatic colorectal cancer. At the time of presentation, DNA from the primary tumor is used to identify the baseline mutation profile; in this case, the tumor is found to be *APC* mutant and *KRAS* wild type (WT). At baseline, evaluation of the patient's plasma DNA only identifies *KRAS* WT fragments. This patient is treated with an anti–epidermal growth factor receptor (EGFR) monoclonal antibody, experiences a clinical response, and has a corresponding decrease in *APC* mutation level, further indicating a decrease in tumor burden. Continuous monitoring of plasma DNA shows the emergence of *KRAS* and *NRAS* mutations and/or *MET* amplification, indicative of the emergence of multiple different resistance clones. Clinical resistance becomes manifest at a later time point. Test tubes represent samples of plasma from which circulating free DNA is extracted and used to monitor the presence of cancer-specific aberrations.

More recently, the mechanisms of acquired resistance to anti–epidermal growth factor receptor (EGFR) therapies have been defined in lung and colorectal cancers. Acquired resistance to gefitinb or erlotinib in approximately 50% of patients with lung cancer develops through the emergence of *EGFR* T790M variants.[44,71] The mutation at residue 790 increases the affinity of EGFR for ATP and so outcompetes binding of the inhibitors. These results were initially obtained in examining biopsies from patients who relapsed during anti-EGFR therapy and were later confirmed through analyses of plasma, providing the first example that resistance to targeted therapies of solid tumors can be detected noninvasively in the blood of patients.[74] Analogously, secondary resistance to the EGFR-blocking monoclonal antibodies cetuximab and panitumumab is associated with the emergence of *KRAS* mutations or *MET* amplification (Fig 3).[60,65] Detection of *KRAS* variants in cfDNA of patients receiving anti-EGFR therapies can identify relapse months before radiologic examination (Fig 3). In this case, the discovery of the mechanisms of resistance to the targeted agent was simultaneously accomplished in tissue and liquid biopsies.[60,65] Another noteworthy aspect of these studies was the emergence of multiple different resistance mutations in the same patient.

These studies were based on candidate gene analyses and required prior knowledge of the mechanisms of resistance. Considering the accessibility of plasma from patients undergoing therapy, it is likely that future studies tracking the emergence of resistant clones will be unbiased. For example, an unbiased approach has been applied to study the acquisition of secondary resistance and to monitor clonal evolution in the blood of patients. In a comprehensive study by Murtaza et al,[44] it was shown that genetic markers of resistance can be noninvasively tracked throughout the course of treatment in breast, ovarian, and lung cancers.

Analysis of ctDNA in plasma samples obtained before and after treatment can ultimately provide a global picture of the molecular genetics of a patient's tumor. This genetic picture includes the dynamic changes in the mutation profile that occur during therapy as well as the heterogeneity that emerges as a result of this therapeutic selective pressure. This understanding of the mechanisms of acquired resistance to targeted agents at the molecular level can be used to plan combinatorial treatments with drugs that will suppress the expansion of the clones that are responsible for most of the current failures of medical treatment (Fig 3). This knowledge could result in the early adoption of alternate therapies before clinical resistance is detected.

NOVEL ADAPTATIONS

Changes in the DNA sequence (eg, somatic point mutations or deletions) are the most frequent class of variants associated with the development of solid tumors and span common oncogenic events, such as *KRAS*, *PIK3CA*, *EGFR*, and *BRAF* mutations.[63] In addition to point mutations, there are several other alterations in

© 2014 by American Society of Clinical Oncology

NeoGenomics
Exhibit 1092 Page 13

nucleic acids present in cancer cells, including amplifications, rearrangements, methylated DNA, mutant mitochondrial DNA, and aneuploidy.[63,75-79] Many have been successfully detected in circulation in patients with cancer, and these studies are further described in our article (Fig 2).

CHROMOSOMAL ALTERATIONS: AMPLIFICATIONS, REARRANGEMENTS, AND ANEUPLOIDY

In addition to using somatic point mutations as markers for the detection of tumor DNA, strategies to detect tumor-derived rearrangements and chromosomal copy number changes (ie, amplifications) in the plasma of patients with cancer have been developed (Fig 2).[41,45,79-81] Two novel methods to quantitate levels of tumor DNA in circulation include personalized analysis of rearranged ends (PARE) and digital karyotyping.[41,45,82] PARE is an approach to identifying DNA rearrangements in human tumors and using these alterations for development of tumor biomarkers. Digital karyotyping is a genome-wide method for detection of copy number alterations associated with such chromosomal changes.[83] Initial analyses have demonstrated that the sensitivity of this approach (ie, ability to detect tumor DNA in a mixture of tumor and normal DNA) is lower than 0.001%. Furthermore, this approach can be modified to detect amplifications across the genome based on the fact that amplified segments of genomic DNA result in chromosomal rearrangements.[41,45] Therefore, when whole-genome sequencing of plasma DNA is performed, paired-end sequence data may reveal aberrantly mapped paired-end reads. The breakpoints of these regions are mapped to genes of interest. Tag counts in the aberrantly mapped regions and pooled normal plasma from healthy individuals are then compared to determine copy number variations in amplified regions.

Approaches like PARE and digital karyotyping using NGS allow for detection of tumor-specific rearrangements, amplification, and aneuploidy in circulation. If initially identified in tumor tissue, these alterations can be tracked in the blood much like point mutations, and levels in ctDNA correspond with tumor burden. Furthermore, these approaches can be used in a blind fashion as liquid biopsies to detect tumor-derived rearrangements and amplifications for genotyping purposes. For example, *HER2/neu* (ERBB2) amplifications or ALK rearrangements could be detected using blood samples without the need to examine tumor tissue for these purposes.[45] Although the cost of such approaches is high at this time, as the price of NGS decreases, these types of analyses will become more readily available for investigative and clinical purposes.

EPIGENETIC CHANGES

Several reports have demonstrated that methylation changes present in the tumor genome are also present in the DNA fragments detected in circulation. Techniques such as methyl-BEAMing, methylation-specific PCR, and quantum dot–based fluorescence resonance energy transfer (QD-FRET) have been adapted to detect methylated fragments of DNA extracted from plasma.[78,84-86] Much like measuring mutations in ctDNA, methylated DNA fragments from the tumor found in circulation are similar to the degree of methylation in the tumor itself and may also correspond to tumor burden. The overall specificity of measuring methylated DNA is lower as compared with genomic alterations, potentially because of the fact that there are often overlapping methylation changes in the tumor and surrounding normal tissue.[78] Furthermore, methylation is not entirely a tumor-specific process; the epigenetic regulation present in the tumor may be active in other nontumor tissue, and often changes increase in frequency in an age-dependent fashion. Accordingly, it is imperative to select the appropriate candidate genes if DNA methylation is to be used as a circulating biomarker. Even with these differences compared with mutations, methylation changes in ctDNA can be useful markers of dynamic changes in tumor burden and noninvasively identify methylation changes that predominate in the tumor. Interestingly, despite the lower specificity, the sensitivity of methylated ctDNA is higher even for early-stage disease, because DNA methylation is often an early event in carcinogenesis. This characteristic of DNA methylation in cancer may speak to its potential use as a screening tool for early detection of malignancy.[78]

DISCUSSION

The analysis of circulating tumor DNA is a promising area of investigation that allows for interrogation of tumor-specific molecular alterations in the circulation. One of the key advantages of ctDNA analyses is the high degree of specificity offered, because mutations found in cfDNA are in essence integral agents of an individual's cancer and are defined by their presence in tumor DNA and absence in matched normal DNA. In terms of sensitivity, levels of ctDNA are abundant in most patients with advanced cancer, allowing for the assessment of molecular heterogeneity, monitoring of tumor dynamics, identification of genetic determinants for therapy, tracking of genomic evolution, and development of acquired resistance. However, more research is needed to establish if these applications will be feasible in tumor types beyond those already studied. In scenarios where only low levels of ctDNA are present (eg, early-stage disease and minimal residual disease), novel genomic methodologies, based on digital PCR, have made such applications possible. New approaches using NGS have increased the throughput necessary for detection of chromosomal abnormalities in ctDNA and will open up the possibility of screening for ctDNA as a tool for the early detection of cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

 © 2014 by American Society of Clinical Oncology

NeoGenomics
Exhibit 1092 Page 14

Employment or Leadership Position: Luis A. Diaz Jr, Personal Genome Diagnostics (U) **Consultant or Advisory Role:** Luis A. Diaz Jr, Amgen (C) **Stock Ownership:** Luis A. Diaz Jr, Personal Genome Diagnostics **Honoraria:** None **Research Funding:** None **Expert Testimony:** None **Patents:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Financial support: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Mandel P, Metais P: Les acides nucleiques du plasma sanguin chez l'homme [in French]. C R Seances Soc Biol Fil 142:241-243, 1948
2. Lo YM, Chiu RW: Genomic analysis of fetal nucleic acids in maternal blood. Annu Rev Genomics Hum Genet 13:285-306, 2012
3. Lo YM, Corbetta N, Chamberlain PF, et al: Presence of fetal DNA in maternal plasma and serum. Lancet 350:485-487, 1997
4. Lo YM, Hjelm NM, Fidler C, et al: Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med 339:1734-1738, 1998
5. Papageorgiou EA, Karagrigoriou A, Tsaliki E, et al: Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21. Nat Med 17:510-513, 2011
6. Fan HC, Blumenfeld YJ, Chitkara U, et al: Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci U S A 105:16266-16271, 2008
7. Beiter T, Fragasso A, Hudemann J, et al: Short-term treadmill running as a model for studying cell-free DNA kinetics in vivo. Clin Chem 57:633-636, 2011
8. Atamaniuk J, Kopecky C, Skoupy S, et al: Apoptotic cell-free DNA promotes inflammation in haemodialysis patients. Nephrol Dial Transplant 27:902-905, 2012
9. García Moreira V, de la Cera Martínez T, Gago González E, et al: Increase in and clearance of cell-free plasma DNA in hemodialysis quantified by real-time PCR. Clin Chem Lab Med 44:1410-1415, 2006
10. Korabecna M, Opatrna S, Wirth J, et al: Cell-free plasma DNA during peritoneal dialysis and hemodialysis and in patients with chronic kidney disease. Ann N Y Acad Sci 1137:296-301, 2008
11. Tovbin D, Novack V, Wiessman MP, et al: Circulating cell-free DNA in hemodialysis patients predicts mortality. Nephrol Dial Transplant 27:3929-3935, 2012
12. Antonatos D, Patsilinakos S, Spanodimos S, et al: Cell-free DNA levels as a prognostic marker in acute myocardial infarction. Ann N Y Acad Sci 1075:278-281, 2006
13. Chang CP, Chia RH, Wu TL, et al: Elevated cell-free serum DNA detected in patients with myocardial infarction. Clin Chim Acta 327:95-101, 2003
14. Destouni A, Vrettou C, Antonatos D, et al: Cell-free DNA levels in acute myocardial infarction patients during hospitalization. Acta Cardiol 64:51-57, 2009
15. Jing RR, Wang HM, Cui M, et al: A sensitive method to quantify human cell-free circulating DNA in blood: Relevance to myocardial infarction screening. Clin Biochem 44:1074-1079, 2011
16. Shimony A, Zahger D, Gilutz H, et al: Cell free DNA detected by a novel method in acute ST-elevation myocardial infarction patients. Acute Card Care 12:109-111, 2010
17. Diehl F, Schmidt K, Choti MA, et al: Circulating mutant DNA to assess tumor dynamics. Nat Med 14:985-990, 2008
18. Macher H, Egea-Guerrero JJ, Revuelto-Rey J, et al: Role of early cell-free DNA levels decrease as a predictive marker of fatal outcome after severe traumatic brain injury. Clin Chim Acta 414:12-17, 2012
19. Ohayon S, Boyko M, Saad A, et al: Cell-free DNA as a marker for prediction of brain damage in traumatic brain injury in rats. J Neurotrauma 29:261-267, 2012
20. Tsai NW, Lin TK, Chen SD, et al: The value of serial plasma nuclear and mitochondrial DNA levels in patients with acute ischemic stroke. Clin Chim Acta 412:476-479, 2011
21. Diehl F, Li M, Dressman D, et al: Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci U S A 102:16368-16373, 2005
22. Holdhoff M, Schmidt K, Donehower R, et al: Analysis of circulating tumor DNA to confirm somatic KRAS mutations. J Natl Cancer Inst 101:1284-1285, 2009
23. Vogelstein B, Kinzler KW: Digital PCR. Proc Natl Acad Sci U S A 96:9236-9241, 1999
24. Dressman D, Yan H, Traverso G, et al: Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. Proc Natl Acad Sci U S A 100:8817-8822, 2003
25. Liu Q, Sommer SS: Pyrophosphorolysis-activated polymerization (PAP): application to allele-specific amplification. Biotechniques 29:1072-1076, 1078, 1080, 2000
26. Daniotti M, Vallacchi V, Rivoltini L, et al: Detection of mutated BRAFV600E variant in circulating DNA of stage III-IV melanoma patients. Int J Cancer 120:2439-2444, 2007
27. Kimura H, Kasahara K, Kawaishi M, et al: Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer. Clin Cancer Res 12:3915-3921, 2006
28. Shinozaki M, O'Day SJ, Kitago M, et al: Utility of circulating B-RAF DNA mutation in serum for monitoring melanoma patients receiving biochemotherapy. Clin Cancer Res 13:2068-2074, 2007
29. Morgan SR, Whiteley J, Donald E, et al: Comparison of KRAS mutation assessment in tumor DNA and circulating free DNA in plasma and serum samples. Clin Med Insights Pathol 5:15-22, 2012
30. Maheswaran S, Sequist LV, Nagrath S, et al: Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med 359:366-377, 2008
31. Kuang Y, Rogers A, Yeap BY, et al: Noninvasive detection of EGFR T790M in gefitinib or erlotinib resistant non-small cell lung cancer. Clin Cancer Res 15:2630-2636, 2009
32. Punnoose EA, Atwal S, Liu W, et al: Evaluation of circulating tumor cells and circulating tumor DNA in non-small cell lung cancer: Association with clinical endpoints in a phase II clinical trial of pertuzumab and erlotinib. Clin Cancer Res 18:2391-2401, 2012
33. Jänne PA, Borras AM, Kuang Y, et al: A rapid and sensitive enzymatic method for epidermal growth factor receptor mutation screening. Clin Cancer Res 12:751-758, 2006
34. Milbury CA, Li J, Makrigiorgos GM: PCR-based methods for the enrichment of minority alleles and mutations. Clin Chem 55:632-640, 2009
35. Newton CR, Graham A, Heptinstall LE, et al: Analysis of any point mutation in DNA: The amplification refractory mutation system (ARMS). Nucleic Acids Res 17:2503-2516, 1989
36. Ogino S, Kawasaki T, Brahmandam M, et al: Sensitive sequencing method for KRAS mutation detection by pyrosequencing. J Mol Diagn 7:413-421, 2005
37. Forshew T, Murtaza M, Parkinson C, et al: Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med 4:136ra68, 2012
38. Li M, Diehl F, Dressman D, et al: BEAMing up for detection and quantification of rare sequence variants. Nat Methods 3:95-97, 2006
39. Higgins MJ, Jelovac D, Barnathan E, et al: Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. Clin Cancer Res 18:3462-3469, 2012
40. Kinde I, Wu J, Papadopoulos N, et al: Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci U S A 108:9530-9535, 2011
41. Leary RJ, Kinde I, Diehl F, et al: Development of personalized tumor biomarkers using massively parallel sequencing. Sci Transl Med 2:20ra14, 2010
42. Taly V, Pekin D, Benhaim L, et al: Multiplex picodroplet digital PCR to detect KRAS mutations in circulating DNA from the plasma of colorectal cancer patients. Clin Chem [epub ahead of print on August 12, 2013]
43. Dawson SJ, Tsui DW, Murtaza M, et al: Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 368:1199-1209, 2013
44. Murtaza M, Dawson SJ, Tsui DW, et al: Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 497:108-112, 2013
45. Leary RJ, Sausen M, Kinde I, et al: Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. Sci Transl Med 4:162ra154, 2012
46. Delgado PO, Alves BC, Gehrke Fde S, et al: Characterization of cell-free circulating DNA in plasma in patients with prostate cancer. Tumour Biol 34:983-986, 2013
47. Hashad D, Sorour A, Ghazal A, et al: Free circulating tumor DNA as a diagnostic marker for breast cancer. J Clin Lab Anal 26:467-472, 2012
48. No JH, Kim K, Park KH, et al: Cell-free DNA level as a prognostic biomarker for epithelial ovarian cancer. Anticancer Res 32:3467-3471, 2012
49. Park JL, Kim HJ, Choi BY, et al: Quantitative analysis of cell-free DNA in the plasma of gastric cancer patients. Oncol Lett 3:921-926, 2012

© 2014 by American Society of Clinical Oncology

NeoGenomics
Exhibit 1092 Page 15

50. Salvianti F, Pinzani P, Verderio P, et al: Multiparametric analysis of cell-free DNA in melanoma patients. PLoS One 7:e49843, 2012

51. Schwarzenbach H, Alix-Panabières C, Müller I, et al: Cell-free tumor DNA in blood plasma as a marker for circulating tumor cells in prostate cancer. Clin Cancer Res 15:1032-1038, 2009

52. Schwarzenbach H, Müller V, Milde-Langosch K, et al: Evaluation of cell-free tumour DNA and RNA in patients with breast cancer and benign breast disease. Mol Biosyst 7:2848-2854, 2011

53. Schwarzenbach H, Stoehlmacher J, Pantel K, et al: Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. Ann N Y Acad Sci 1137:190-196, 2008

54. Fleischhacker M, Schmidt B: Circulating nucleic acids (CNAs) and cancer: A survey. Biochim Biophys Acta 1775:181-232, 2007

55. Jahr S, Hentze H, Englisch S, et al: DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 61:1659-1665, 2001

56. Stroun M, Lyautey J, Lederrey C, et al: About the possible origin and mechanism of circulating DNA: Apoptosis and active DNA release. Clin Chim Acta 313:139-142, 2001

57. Wyllie AH: Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284:555-556, 1980

58. Mouliere F, Robert B, Arnau Peyrotte E, et al: High fragmentation characterizes tumour-derived circulating DNA. PLoS One 6:e23418, 2011

59. Chan KC, Zhang J, Hui AB, et al: Size distributions of maternal and fetal DNA in maternal plasma. Clin Chem 50:88-92, 2004

60. Misale S, Yaeger R, Hobor S, et al: Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 486:532-536, 2012

61. Overman MJ, Modak J, Kopetz S, et al: Use of research biopsies in clinical trials: Are risks and benefits adequately discussed? J Clin Oncol 31:17-22, 2013

62. Gerlinger M, Rowan AJ, Horswell S, et al: Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 366:883-892, 2012

63. Vogelstein B, Papadopoulos N, Velculescu VE, et al: Cancer genome landscapes. Science 339:1546-1558, 2013

64. De Mattos-Arruda L, Cortes J, Santarpia L, et al: Circulating tumour cells and cell-free DNA as tools for managing breast cancer. Nat Rev Clin Oncol 10:377-389, 2013

65. Diaz LA Jr, Williams RT, Wu J, et al: The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 486:537-540, 2012

66. Ito K, Hibi K, Ando H, et al: Usefulness of analytical CEA doubling time and half-life time for overlooked synchronous metastases in colorectal carcinoma. Jpn J Clin Oncol 32:54-58, 2002

67. Yoshimasu T, Maebeya S, Suzuma T, et al: Disappearance curves for tumor markers after resection of intrathoracic malignancies. Int J Biol Markers 14:99-105, 1999

68. Riedinger JM, Wafflart J, Ricolleau G, et al: CA 125 half-life and CA 125 nadir during induction chemotherapy are independent predictors of epithelial ovarian cancer outcome: Results of a French multicentric study. Ann Oncol 17:1234-1238, 2006

69. Bidard FC, Madic J, Mariani P, et al: Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastatic uveal melanoma. Int J Cancer [epub ahead of print on August 12, 2013]

70. Goncalves R, Bose R: Using multigene tests to select treatment for early-stage breast cancer. J Natl Compr Canc Netw 11:174-182, 2013; quiz 182

71. Pao W, Miller VA, Politi KA, et al: Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2:e73, 2005

72. Antonescu CR, Besmer P, Guo T, et al: Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. Clin Cancer Res 11:4182-4190, 2005

73. Branford S, Rudzki Z, Walsh S, et al: Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 102:276-283, 2003

74. Taniguchi K, Uchida J, Nishino K, et al: Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas. Clin Cancer Res 17:7808-7815, 2011

75. Azad N, Zahnow CA, Rudin CM, et al: The future of epigenetic therapy in solid tumours: Lessons from the past. Nat Rev Clin Oncol 10:256-266, 2013

76. He Y, Wu J, Dressman DC, et al: Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. Nature 464:610-614, 2010

77. Holland AJ, Cleveland DW: Boveri revisited: Chromosomal instability, aneuploidy and tumorigenesis. Nat Rev Mol Cell Biol 10:478-487, 2009

78. Li M, Chen WD, Papadopoulos N, et al: Sensitive digital quantification of DNA methylation in clinical samples. Nat Biotechnol 27:858-863, 2009

79. Beck J, Urnovitz HB, Mitchell WM, et al: Next generation sequencing of serum circulating nucleic acids from patients with invasive ductal breast cancer reveals differences to healthy and nonmalignant controls. Mol Cancer Res 8:335-342, 2010

80. McBride DJ, Orpana AK, Sotiriou C, et al: Use of cancer-specific genomic rearrangements to quantify disease burden in plasma from patients with solid tumors. Genes Chromosomes Cancer 49:1062-1069, 2010

81. Chan KC, Jiang P, Zheng YW, et al: Cancer genome scanning in plasma: Detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing. Clin Chem 59:211-224, 2013

82. Wang TL, Diaz LA Jr, Romans K, et al: Digital karyotyping identifies thymidylate synthase amplification as a mechanism of resistance to 5-fluorouracil in metastatic colorectal cancer patients. Proc Natl Acad Sci U S A 101:3089-3094, 2004

83. Wang TL, Maierhofer C, Speicher MR, et al: Digital karyotyping. Proc Natl Acad Sci U S A 99:16156-16161, 2002

84. Weaver KD, Grossman SA, Herman JG: Methylated tumor-specific DNA as a plasma biomarker in patients with glioma. Cancer Invest 24:35-40, 2006

85. Bailey VJ, Keeley BP, Zhang Y, et al: Enzymatic incorporation of multiple dyes for increased sensitivity in QD-FRET sensing for DNA methylation detection. Chembiochem 11:71-74, 2010

86. Bailey VJ, Zhang Y, Keeley BP, et al: Single-tube analysis of DNA methylation with silica superparamagnetic beads. Clin Chem 56:1022-1025, 2010

 © 2014 by American Society of Clinical Oncology

NeoGenomics
Exhibit 1092 Page 16