HealthCare
MARKEY CANCER CENTER
An NCI-Designated Cancer Center

Navigating from Somatic Tumor Testing to Germline Genetic Testing

Justine M. Cooper, MS, CGC
Certified Genetic Counselor
UK Markey Cancer Center

Somatic Testing vs. Germline Testing

- Somatic
  - Identifies mutations in the tumor (ie. acquired changes)
  - Performed on tumor tissue
  - Patient has cancer
  - Purpose is to identify treatment options, determine prognosis
  - Ordered by oncologist
  - Patient not often consented
- Germline
  - Identifies mutations in the germline (ie. mutations you are born with)
  - Performed on blood/saliva
  - Patient may be unaffected
  - Purpose is to identify patients with inherited cancer predisposition syndromes
  - Often ordered by GC, sometimes by oncologist, surgeon, PCP, etc.
  - Patient often receives counseling

What genes are analyzed?

ABL1	ABL2	AKT1	AKT2	AKT3	ALK	APC	AR	ARAF	ARID1A
ARNTL	ASXL1	ATM	ATR	ATRX	AURKA	BAP1	BARD1	BCL2	BCL6
BCOR	BCORL1	BIRC3	BRAF	BRCA1	BRCA2	BTK	CALR	CARD11	CBL
CBLB	CCND1	CCND2	CCND3	CCNE1	CD79B	CDH1	CDK12	CDK4	CDK6
CDKN1A	CDKN1B	CDKN2A	CDKN2B	CDKN2C	CEBPA	CIC	CLSTN1	CREBBP	CRLF2
CSF1R	CSF3R	CTNNB1	CUX1	DDR2	DNAJB1	DNMT3A	EGFR	EP300	EPHA2
ERBB2	ERBB4	ESR1	ETV1	ETV6	EWSR1	EZH2	FAM5C	FBXW7	FGFR1
FGFR2	FGFR3	FLT3	FLT4	FOXL2	FOXO1	FOXP1	FUBP1	GATA1	GATA2
GATA3	GNA11	GNA13	GNAQ	GNAS	HNF1A	HNRNPK	HRAS	ID3	IDH1
IDH2	IKZF1	IL7R	JAK1	JAK2	JAK3	KDM6A	KDR	KEAP1	KIF17
KIT	KLHL6	KMT2A	KMT2C	KMT2D	KRAS	MAP2K1	MAP2K2	MCL1	MEF2B
MET	MPL	MTOR	MYBL2	MYD88	NF1	NF2	NFE2L2	NOTCH1	NOTCH2
NOTCH3	NPM1	NRAS	NSD1	NT5C2	NTRK1	NTRK2	NTRK3	PAWR	PAX5
PBRM1	PDGFRA	PDGFRB	PHF6	PIAS2	PIK3C2A	PIK3C2B	PIK3CA	PIK3CB	PIK3CG
PIK3R1	PIK3R2	PIK3R5	PLCG2	PRDM1	PRKACA	PRMT5	PTCH1	PTEN	PTPN11
PTPRD	PTPRT	RAC1	RAD21	RAF1	RB1	RELB	RET	RHEB	RHOA
RIT1	ROS1	RPS6KB1	RUNX1	SETBP1	SF3B1	SH2B3	SHH	SMAD4	SMARCB1
SMC1A	SMC3	SMO	SOCS1	SOD2	SRC	SRSF2	STAG2	STAT3	STK11
SUFU	SUZ12	TCF3	TERT	TET1	TET2	TMC6	TNFAIP3	TNFRSF14	TP53
TSC1	TSC2	U2AF1	VHL	WHSC1	WNT1	WT1	ZRSR2		

Genes in gray are the 94 included in the Hematologic Cancer Panel.

FOUNDATIONONE

MI PROFILE
MOLECULAR INTELLIGENCE TUMOR REPORT

CARIS LIFE SCIENCES

SUMMARY OF RESULTS (SEE APPENDIX FOR FULL DETAILS)

Assay	Result
Microsatellite Instability (MSI)	Stable
Total Mutation	

Biomarker		
ABL1		
AKT1		
AKT2		
ALK		
APC		
AR		
ARAF		
ARID1A		
ATM		
AURKB	NGS	Amplifica
BAP1	NGS	Mutation
BMPR1A	NGS	Mutation
BRAF	NGS	Mutated, Exon 15 |
BRCA1	NGS	Mutation
BRCA2	NGS	Mutation
c-KIT	NGS	Mutation
CCND1	NGS	Amplifica
CCND3	NGS	Amplifica
CCNE1	NGS	Amplifica
CDC73	NGS	Mutation
CDH1	NGS	Mutation
CDK4	NGS	Mutation
	NGS	Amplifica
CDK6	NGS	Amplifica
CDK8	NGS	Amplifica
CDKN1B	NGS	Mutation
CDKN2A	NGS	Mutation
	NGS	Amplification Not Detected
FH	NGS	Mutation Not Detected

Paradigm

Inivata

*Exons selected to maximize detection of known somatic mutations. List availabl

** includes TERT pro

IHC: Immunohistochemistry CISH: Chromogenic *in situ* hybridization NGS: Next-Generation Sequencing

Biomarker Results continued on the next page. >

PATIENT: Patient, Test (XX-Mon-19XX) **TN16-XXXXXX** **PHYSICIAN:** Ordering Phyician, MD

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • 3531.01 • Zoran Gatalica, MD, DSc, Medical Director • ©2016 Caris Life Sciences. All rights reserved. Page 2 of 14

MI PROFILE
MOLECULAR INTELLIGENCE TUMOR REPORT

CARIS LIFE SCIENCES

SUMMARY OF RESULTS (SEE APPENDIX FOR FULL DETAILS)

Biomarker	Method	Result	Biomarker	Method	Result
FLCN	NGS	Mutation Not Detected	MUTYH	NGS	Mutation Not Detected
	NGS	Mutation Not Detected	MYC	NGS	Amplification Not Detected
					Mutation Not Detected
					Mutation Not Detected
					Amplification Not Detected
					Amplification Not Detected
					Mutation Not Detected
					Mutation Not Detected
					Mutation Not Detected

T360 Panel

of Covered Exons*

cynvenio™

F1	MLH1	MTOR	KRAS	MET
DGFRA	NF1	PDGFRA	MYC	PDGFRA
HER	PTEN	RB1	PIK3CA	RAF1

Mutation Not Detected
Mutation Not Detected
Negative | 0, 100%
Mutation Not Detected
Mutation Not Detected
ve | 0, 100%
on Not Detected
on Not Detected
on Not Detected
on Not Detected
cation Not Detected
on Not Detected
cation Not Detected
on Not Detected
cation Not Detected

PGD
Personal Genome Diagnostics

Biomarker Results continued on the next page. >

PATIENT: Patient, Test (XX-Mon-19XX) **TN16-XXXXXX** **PHYSICIAN:** Ordering Phyician, MD

4610 South 44th Place, Suite 100 • Phoenix, AZ 85040 • (888) 979-8669 • Fax: (866) 479-4925
CLIA 03D1019490 • CAP 7195577 • ISO 15189:2012 • 3531.01 • Zoran Gatalica, MD, DSc, Medical Director • ©2016 Caris Life Sciences. All rights reserved. Page 3 of 14

SAMPLE REPORT FOR

by demonstrating high concordance with genomic profiles of ninety four clinical specim

ASCO Policy Statement

ASCO supports the communication to patients of medically relevant incidental germline findings from somatic mutation profiling conducted in the clinical setting. Oncology providers should communicate the potential for incidental and secondary germline information to patients before conducting somatic mutation profiling and should review the potential benefits, limitations, and risks before testing.

VOLUME 33 · NUMBER 31 · NOVEMBER 1 2015

JOURNAL OF CLINICAL ONCOLOGY ASCO SPECIAL ARTICLE

American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility

Mark E. Robson, Angela R. Bradbury, Banu Arun, Susan M. Domchek, James M. Ford, Heather L. Hampel, Stephen M. Lipkin, Sapna Syngal, Dana S. Wollins, and Noralane M. Lindor

See accompanying editorial on page 3533

Mark E. Robson, Memorial Sloan Kettering Cancer Center; Mark E. Robson and Stephen M. Lipkin, Weill Cornell Medical College, New York, NY; Angela R. Bradbury and Susan M. Domchek, Hospital of the University of Pennsylvania, Philadelphia, PA; Banu Arun, MD Anderson Cancer Center, Houston, TX; James M. Ford, Stanford University Medical Center, Stanford, CA; Heather L. Hampel, Ohio State University Comprehensive Cancer Center, Columbus, OH; Sapna Syngal, Dana-Farber Cancer Institute, Boston, MA; Dana S. Wollins, American Society of Clinical Oncology, Alexandria, VA; and Noralane M. Lindor, Mayo Clinic, Scottsdale, AZ.

Published online ahead of print at www.jco.org on August 31, 2015.

Processed as a Rapid Communication manuscript.

Reprint requests: American Society of Clinical Oncology, 2318 Mill Rd, Suite 800, Alexandria, VA 22314; cancerpolicy@asco.org.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

Corresponding author: Mark E. Robson, MD, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065; e-mail: robsonm@mskcc.org.

© 2015 by American Society of Clinical Oncology

0732-183X/15/3331w-3660w/$20.00

DOI: 10.1200/JCO.2015.63.0996

A B S T R A C T

The American Society of Clinical Oncology (ASCO) has long affirmed that the recognition and management of individuals with an inherited susceptibility to cancer are core elements of oncology care. ASCO released its first statement on genetic testing in 1996 and updated that statement in 2003 and 2010 in response to developments in the field. In 2014, the Cancer Prevention and Ethics Committees of ASCO commissioned another update to reflect the impact of advances in this area on oncology practice. In particular, there was an interest in addressing the opportunities and challenges arising from the application of massively parallel sequencing—also known as next-generation sequencing—to cancer susceptibility testing. This technology introduces a new level of complexity into the practice of cancer risk assessment and management, requiring renewed effort on the part of ASCO to ensure that those providing care to patients with cancer receive the necessary education to use this new technology in the most effective, beneficial manner. The purpose of this statement is to explore the challenges of new and emerging technologies in cancer genetics and provide recommendations to ensure their optimal deployment in oncology practice. Specifically, the statement makes recommendations in the following areas: germline implications of somatic mutation profiling, multigene panel testing for cancer susceptibility, quality assurance in genetic testing, education of oncology professionals, and access to cancer genetic services.

J Clin Oncol 33:3660-3667. © 2015 by American Society of Clinical Oncology

INTRODUCTION

The American Society of Clinical Oncology (ASCO) is the leading medical professional oncology society committed to conquering cancer through research, education, prevention, and delivery of high-quality patient care. ASCO has long affirmed that the recognition and management of individuals with an inherited susceptibility to cancer are core elements of oncology care. ASCO released its first statement on genetic testing in 1996[1] and updated that statement in 2003 and 2010 in response to developments in the field of clinical cancer genetics.[2,3] In 2014, the Cancer Prevention and Ethics Committees of ASCO commissioned another update to reflect the impact of advances in this area on oncology practice. In particular, ASCO wished to address the opportunities and challenges arising from the application of massively parallel sequencing—also known as next-generation sequencing (NGS)—to cancer susceptibility testing.

NGS is a powerful technology that permits the characterization of large amounts of DNA sequence much quicker and at lower cost than traditional Sanger sequencing.[4-6] The ability to affordably sequence panels of genes, exomes, and even whole genomes presents an enormous opportunity, and investigators in all fields of medicine are exploring how to best use this new tool to improve patient outcomes.[7] In oncology, NGS makes it feasible to catalog the DNA sequence variations within a patient's cancer (ie, somatic mutation profiling), with the goal of defining therapeutic targets and thereby improving patient outcomes through the application of specific therapies directed at those targets. NGS can facilitate the identification of inherited susceptibility to cancer (and other diseases) either in the course of somatic mutation profiling or through direct germline multigene (multiplex) panel testing. These applications of NGS challenge existing paradigms of counseling and testing for inherited susceptibility and raise important questions regarding

HealthCare
MARKEY CANCER CENTER
An NCI-Designated Cancer Center

Ready for Surprises?

NGS offers promise, but poses significant challenges for oncologists who are ill prepared to handle incidental findings that have clinical implications for at-risk family members. This report underscores the need for oncologists to develop a framework for pre- and post-communication of risks to patients undergoing routine tumor-only sequencing

IJC
International Journal of Cancer

Tumor genome analysis includes germline genome: Are we ready for surprises?

Daniel V.T. Catenacci[1], Andrea L. Amico[1], Sarah M. Nielsen[1,2], Daniel M. Geynisman[1], Brittany Rambo[1], George B. Carey[1], Cassandra Gulden[1,2], Jim Fackenthal[1,2], Robert D. Marsh[1], Hedy L. Kindler[1] and Olufunmilayo I. Olopade[1,2]

[1] Section of Hematology/Oncology, Department of Medicine, University of Chicago, Chicago, IL
[2] Center for Clinical Cancer Genetics, Department of Medicine, University of Chicago, Chicago, IL

We sought to describe the spectrum of potential and confirmed germline genomic events incidentally identified during routine medium-throughput somatic tumor DNA sequencing, and to provide a framework for pre- and post-test consent and counseling for patients and families. Targeted tumor-only next-generation sequencing (NGS) had been used to evaluate for possible drug-gable genomic events obtained from consecutive new patients with metastatic gastroesophageal, hepatobiliary or colorectal cancer seen at the University of Chicago. A panel of medical oncologists, cancer geneticists and genetic counselors retrospec-tively grouped these patients ($N = 111$) based on probability of possessing a potentially inherited mutation in a cancer sus-ceptibility gene, both prior to *and* after incorporating tumor-only NGS results. High-risk patients (determined from NGS results) were contacted and counseled in person by a genetic counselor ($N = 21$). When possible and indicated, germline genetic testing was offered. Of 8 evaluable high-risk patients, 7 underwent germline testing. Three (37.5%) had confirmed actionable germline mutations (all in the *BRCA2* gene). NGS offers promise, but poses significant challenges for oncologists who are ill prepared to handle incidental findings that have clinical implications for at risk family members. In this relatively small cohort of patients undergoing tumor genomic testing for gastrointestinal malignancies, we incidentally identified 3 *BRCA2* mutations carriers. This report underscores the need for oncologists to develop a framework for pre- and post-test communication of risks to patients undergoing routine tumor-only sequencing.

We have reached a critical point in our technological evolu-tion whereby our ability to amass large amounts of genetic information has far surpassed our experience and expertise regarding the clinical application of the derived material. Never has this discrepancy been more magnified—nor have our limitations been so apparent—as with the application of next-generation sequencing (NGS) technology to modern-day oncology practice, where decisions regarding cancer care are increasingly being driven by data derived from NGS.[1–4]

The significant challenges associated with implementing NGS into routine multiplex testing of germline DNA in indi-viduals who are determined to have sufficient family risk *via* traditional clinical cancer genetics models have recently been summarized by Domchek *et al.*[5] In contrast to the established model of "à la carte" gene sequencing in serial fashion, guided by personal and family history, age at diagnosis and disease histology, we now have the ability to evaluate hun-dreds to thousands of genes simultaneously—for better or worse. While this may have the advantage of being expedient and potentially cost-effective, particularly when there is no clear pattern attributable to a given genetic syndrome, we are often left with a deluge of information, yet with no guidelines for post-NGS counseling or clinical interpretation. Further-more, the ethical and legal ramifications regarding disclosure of genetic information, generated from *coupled* somatic/germ-line NGS testing, to cancer patients and their relatives has been recently outlined by Lolkema *et al.*[6]

However, a more pressing issue in clinical oncology practice is the ever-increasing routine sequencing of tumor DNA alone.[4,7] The results obtained from this approach not only con-tain the intended somatic molecular profile of the tumor, but

Key words: somatic, germline, next generation sequencing, genetic counseling
This is an open access article under the terms of the Creative Com-mons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
Additional Supporting Information may be found in the online version of this article.
Grant sponsor: NIH K12; **Grant number:** CA139160-01A; **Grant sponsor:** NCI P50; **Grant number:** CA125183; **Grant sponsor:** ASCO Young Investigator Award, University of Chicago Comprehensive Cancer Center Pilot Award for Precision Oncology, Live Like Katie Foundation Award for Pancreatobiliary Cancers and Cancer Research Foundation Young Investigator Award, The Ralph and Marion Falk Medical Research Trust and the American Cancer Society
DOI: 10.1002/ijc.29128
History: Received 26 Mar 2014; Accepted 25 July 2014; Online 6 Aug 2014
Correspondence to: Daniel V. T. Catenacci, Section of Hematology/Oncology, University of Chicago Medical Center, 5841 S. Maryland Avenue, MC2115, Chicago, IL 60637, USA, Tel: 773-702-7596, Fax: 773-702-3163, E-mail: dcatenac@medicine.bsd.uchicago.edu

Cancer Genetics

HealthCare
MARKEY CANCER CENTER
An NCI-Designated Cancer Center

Discussion Prior to Testing

Table 3. Recommendations for screening and genetic counseling based on pre- and post-NGS probability risk

Risk group based on Pre-NGS probability	Description of Pre-NGS groups	Recommendations to the oncologist before/after ordering NGS
High	• Strong family or personal history of malignancy, per current tumor-specific genetic counseling guidelines • Ashkenazi Jewish heritage	• Emphasize the implications of NGS testing, including the possibility of identifying a somatic mutation that would be suspicious for germline potential. • **Prior to testing:** ask the patient about their preferences regarding disclosure of this information. • **Prior to obtaining NGS results:** strongly consider referral to a genetic counselor.
Intermediate	• May have family history of malignancy or other high risk features (e.g. very early age at diagnosis), but does not meet current guidelines for referral to genetic counseling/testing.	• Discuss the implications of NGS testing and the possibility of identifying a somatic mutation that would be suspicious for germline potential. • **Prior to testing:** ask the patient about their preferences regarding disclosure of this information. • **After NGS testing:** Use post-NGS risk to determine whether referral to genetic counselor and germline testing is warranted. • When in doubt, discuss the case with a genetic counselor to clarify whether referral is recommended.
Low	• Unimpressive family history (either no known history of malignancy or remote, isolated cases)	• Briefly mention the implications of NGS testing and the rare possibility of identifying a somatic mutation that would be suspicious for germline potential. • **Prior to testing:** Ask the patient about their preferences regarding disclosure of this information. • **After NGS testing:** Use postNGS risk to determine whether referral to genetic counselor and germline testing is warranted. • When in doubt, discuss the case with a genetic counselor to clarify whether referral is recommended.

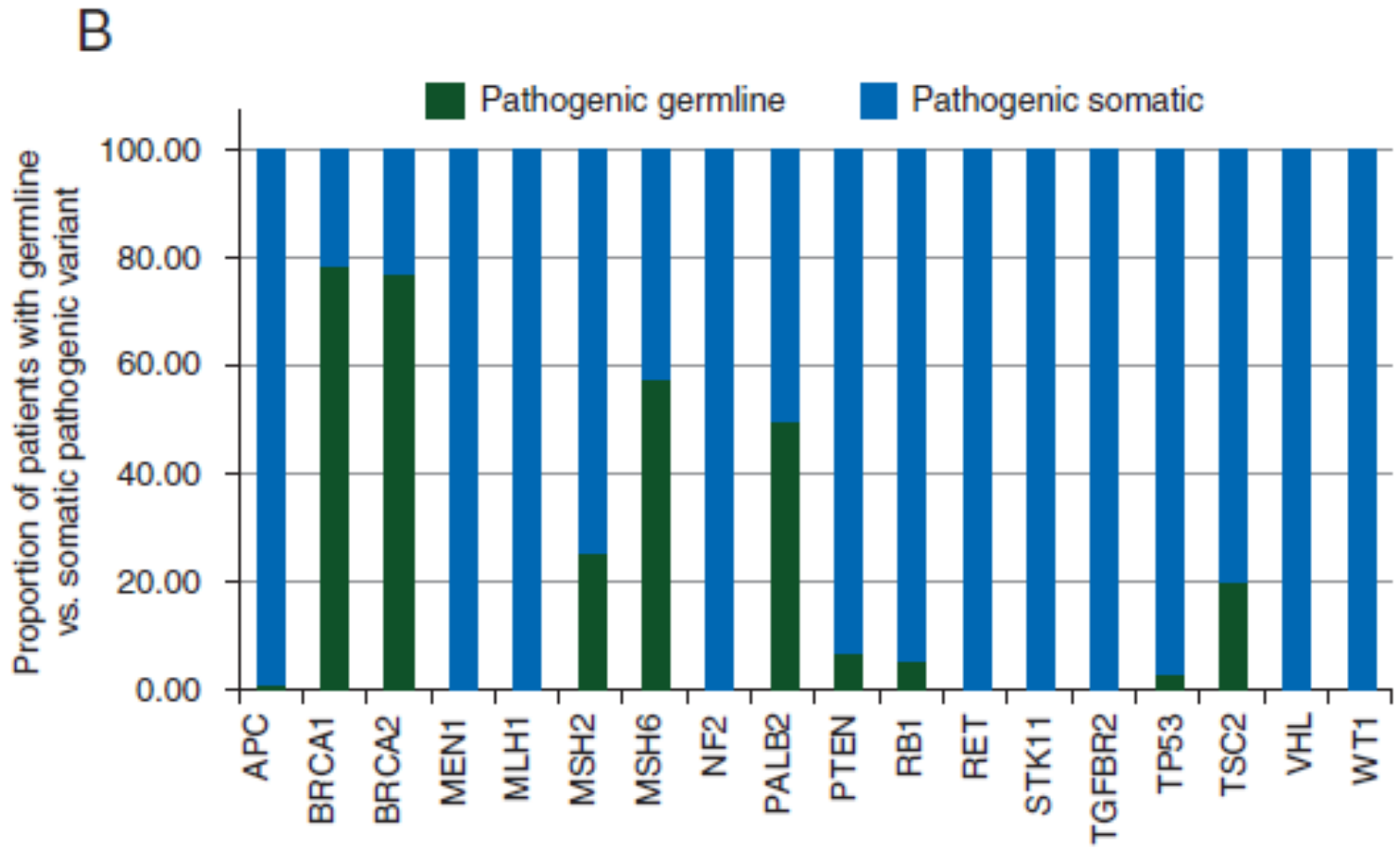
Abbreviation: NGS, next generation sequencing of tumor tissue.

Catenacci et al. 2015

How often are germline findings identified in tumor?

- Meric-Bernstam et al. (2016)
  - 1000 advanced cancer patients offered tumor-normal sequencing with 202-gene panel (19 clinically actionable in germline) at MD Anderson
  - 422/100 (42%) had pathogenic somatic variant in one of 19 genes
  - 43/1000 (4.3%) had a likely pathogenic germline variant identified
  - Tumor types included breast, colon, brain, melanoma, sarcoma, ovary, head and neck
- Schrader et al. (2016)
  - 1566 advanced cancer patients offered tumor-normal sequencing with MSK-IMPACT panel (341-gene panel)
  - 198/1566 (12.6%) had pathogenic germline variant in cancer susceptibility gene
  - Germline findings concordant with cancer type in only 81/198 (40.9%) cases
- Seifert et al. (2016)
  - 439 unselected cancer patients offered tumor-normal sequencing of 247 genes (36 genes strongly associated with hereditary cancer) at UNC
  - 19/439 (4.3%) had pathogenic germline variant
  - 12/19 (63%) were concordant with cancer type

Percentage of Somatic vs. Germline Variants



HealthCare
MARKEY CANCER CENTER
An NCI-Designated Cancer Center

Meric-Bernstam et al.

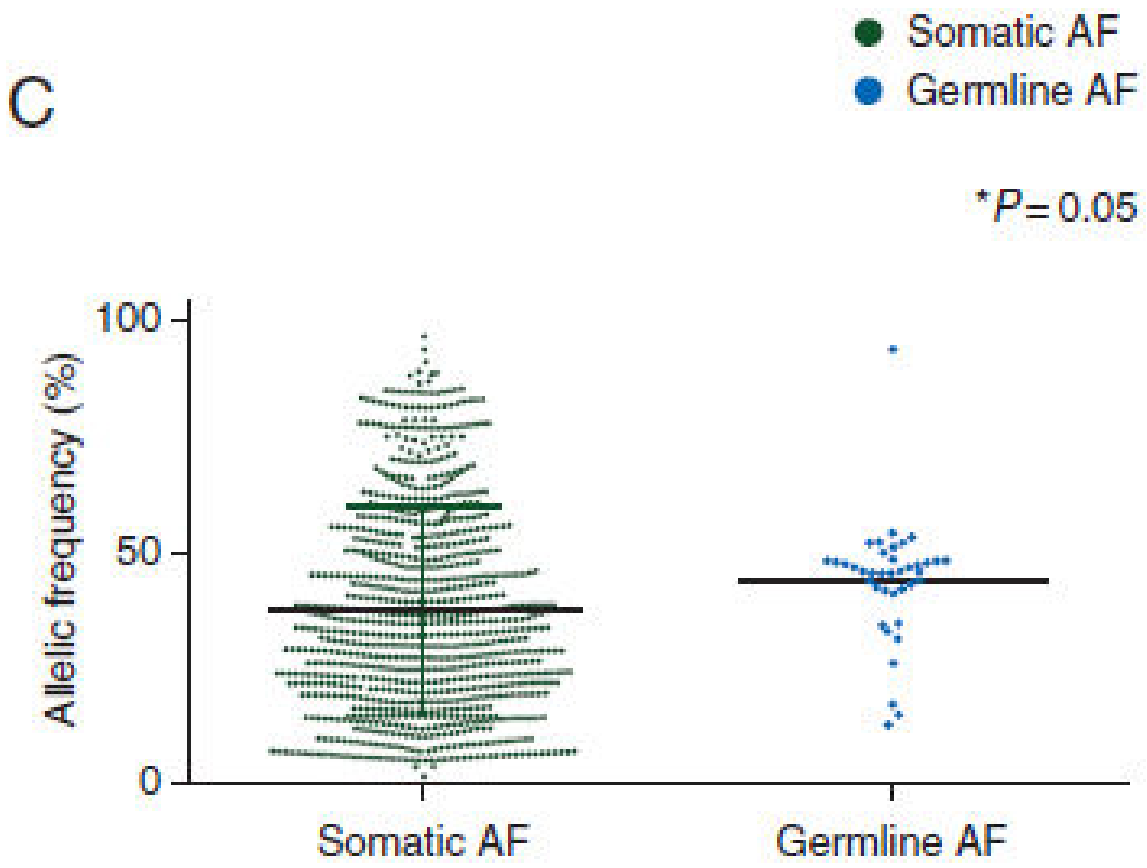
Suggestive of Germline Finding

- All BRCA1 and BRCA2 pathogenic variants regardless of tumor type (NCCN guideline)
- Founder mutations (ie. MSH2 exon 1-6 deletion, TP53 R337H)
- Uncommonly somatically mutated genes (ie. CHEK2, PALB2)
- Gene consistent with phenotype
- Same mutation detected in multiple primary tumors
- Underlying mutation pattern (ie. hypermutated tumor)
- High mutant allele frequency (MAF)

Mutant Allele Frequency

- Mutant allele frequency (MAF) can be suggestive of a germline mutation
- MAF >50% suggest loss of heterozygosity (LOH)
- Germline mutations in tumor suppressor genes often undergo LOH events
- High MAF also seen in normal course of tumor development without a germline mutation

Mutant Allele Frequencies



Do not use MAF to rule OUT a germline mutation!

HealthCare
MARKEY CANCER CENTER
An NCI-Designated Cancer Center

Meric-Bernstam et al.

Refer If Tumor Testing Is Normal?

- Regardless of tumor results, if the patient meets criteria for germline testing (NCCN guidelines), REFER!
  - Large deletion in somatic can mask germline point mutation
  - Somatic vs. germline labs cover different areas of the genes
  - Pathogenic variant in germline may not be considered pathogenic in somatic, therefore not reported
  - Not all hereditary cancer genes are on tumor panels

Considerations for incidental findings

- Insurance coverage
- Single-site vs. full panel
- Patients confused about germline vs. somatic testing
- Patient previously declined counseling/testing
- Sick patients
  - Need to be seen relatively quickly
  - May not directly impact patient
  - Who do we disclose results to?

Markey Cancer Center Genetic Counseling

Questions or want to refer a patient?

Justine M. Cooper, MS, CGC

Justine.Cooper@uky.edu

3-3083