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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 druggable 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 retrospectively grouped these patients ($N = 111$) based on probability of possessing a potentially inherited mutation in a cancer susceptibility 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 evolution 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 individuals 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 hundreds 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. Furthermore, the ethical and legal ramifications regarding disclosure of genetic information, generated from *coupled* somatic/germline 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 contain the intended somatic molecular profile of the tumor, but

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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

What's new?
High-throughput, 'next-generation sequencing' (NGS) allows millions of DNA strands to be sequenced in parallel. NGS is increasingly used to test tumors for mutations that may guide therapy. Sometimes, however, this testing can reveal mutations that are known to be inherited, which means that family members are also at increased risk for cancer. How should this information be presented? This article underscores the need for oncologists to develop a framework for pre- and post-test communication and counseling regarding risk for patients undergoing tumor-only sequencing.

also any underlying germline aberrations that may be present, whether or not they were suspected prior to testing. Multiplex NGS of tumors, using "targeted" exon and intron capture (~200–500 genes), is already commercially available, and its routine use is increasing.[7] The intent of multiplex analysis in this setting is to address interpatient heterogeneity from limited tissue samples, and to identify "driver" events that may be "actionable" pharmacologically, thereby increasing therapeutic options for patients, particularly those with access to phase I clinical trials.[2,8–10] The acknowledged barriers that may prevent the realization of this "personalized" genomics-driven approach are numerous, and were recently reviewed.[11–15]

An under-recognized concern of "tumor-only" NGS is the absence of appropriate pretest counseling regarding the potential for discovering underlying germline mutations, and the lack of post-test guidance for both physicians and families who are faced with a previously unrecognized inherited cancer risk. The concerns expressed by Domchek *et al.* regarding select patients seen in high-risk genetic counseling settings are drastically magnified in this situation, as they apply to many more patients and families, yet the information may be conveyed by physicians who are less well-equipped to deliver it. The same cautions and recommendations still apply with respect to high or medium penetrance genes, as well as to variants of unknown significance (VUS).[3,5] Similarly, the issues raised by Lolkema *et al.* are expanded to *all* patients who have NGS somatic tumor testing. Moreover, there is the added complexity in this "tumor-only" scenario when a *possible* (usually unexpected) germline mutation is uncovered, of deciding whether to further investigate these aberrations as merely somatic or indeed germline. Clearly, obtaining simultaneous germline testing would eliminate this dilemma, but this is not current practice for multiple reasons including cost, consent/counseling, and logistics. Therefore tumor-only sequencing has been preferred.

In this report we systematically, retrospectively evaluated a large cohort of gastrointestinal cancer patients who had undergone routine tumor NGS for therapeutic intent. We sought to identify those patients who might need follow-up for unsuspected underlying germline events, and to determine whether we could confirm the "high risk" cases post-NGS as germline carriers. We discuss potential implications of tumor DNA profiling in various pretest probability risk scenarios (as determined by family history, age, etc.), including when the tumor genomic profile included rarely somatically altered genes with clearly characterized germline genotype-phenotype correlations such as *BRCA1/2*. Finally, we provide a framework for pre- and post-test consent and counseling of patients undergoing routine tumor sequencing.

Patients and Methods

Consent was obtained from new patients with gastrointestinal cancers seen in the University of Chicago Gastrointestinal Oncology Clinic between September 2012 and September 2013. Tumors from patients with adequate tissue had undergone targeted gene sequencing using the FoundationOne, NGS assay ($N = 111$).[16] All reports were reviewed by an expert panel of medical oncologists, cancer geneticists and genetic counselors.

Using early age of onset and family history

Patients were roughly grouped into three basic pre-NGS genetic risk categories—high, intermediate and low—based on age at diagnosis and personal/family history. For example, a patient was placed in the "high-risk" group if he/she had strong family history, including more than 2 first-degree relatives, or relatives in successive generations, having either the same cancer diagnosis or cancers which are known to be associated with a cancer susceptibility syndrome (*e.g.*, gastric and colon (Lynch), or breast and gastric (CDH1), etc.). Additionally, patients were stratified in the high-risk group if either their, or a first-degree relative's, cancer diagnosis occurred at a young age (*i.e.*, <60 years), or if either they or a first-degree relative had two known different cancer diagnoses. Individuals were placed in the "intermediate-risk" group if they had a family history of cancer in up to 2 first-degree relatives and/or the ages of cancer diagnosis were characteristic of what would be seen with sporadic cases (*i.e.*, age of onset >60 years). Patients in the "low-risk" group typically had family histories that were unremarkable for cancer diagnoses and with their own cancer diagnosis occurring in the 7th decade of life, or later. Note that these groupings are distinct from the post-NGS categories. In the latter—*i.e.*, the high, intermediate and low, *post-NGS* risk groups—NGS test results were included in the stratification criteria as discussed below.

Using tumor testing results

Patients were roughly grouped based on the tumor NGS testing results to examine liklihood of having a germline mutation. To do this, genes previously associated with inherited cancer susceptibility and included in commercial cancer gene panels such as BROCA, COLOSEQ[17–19] and Ambry Genetics

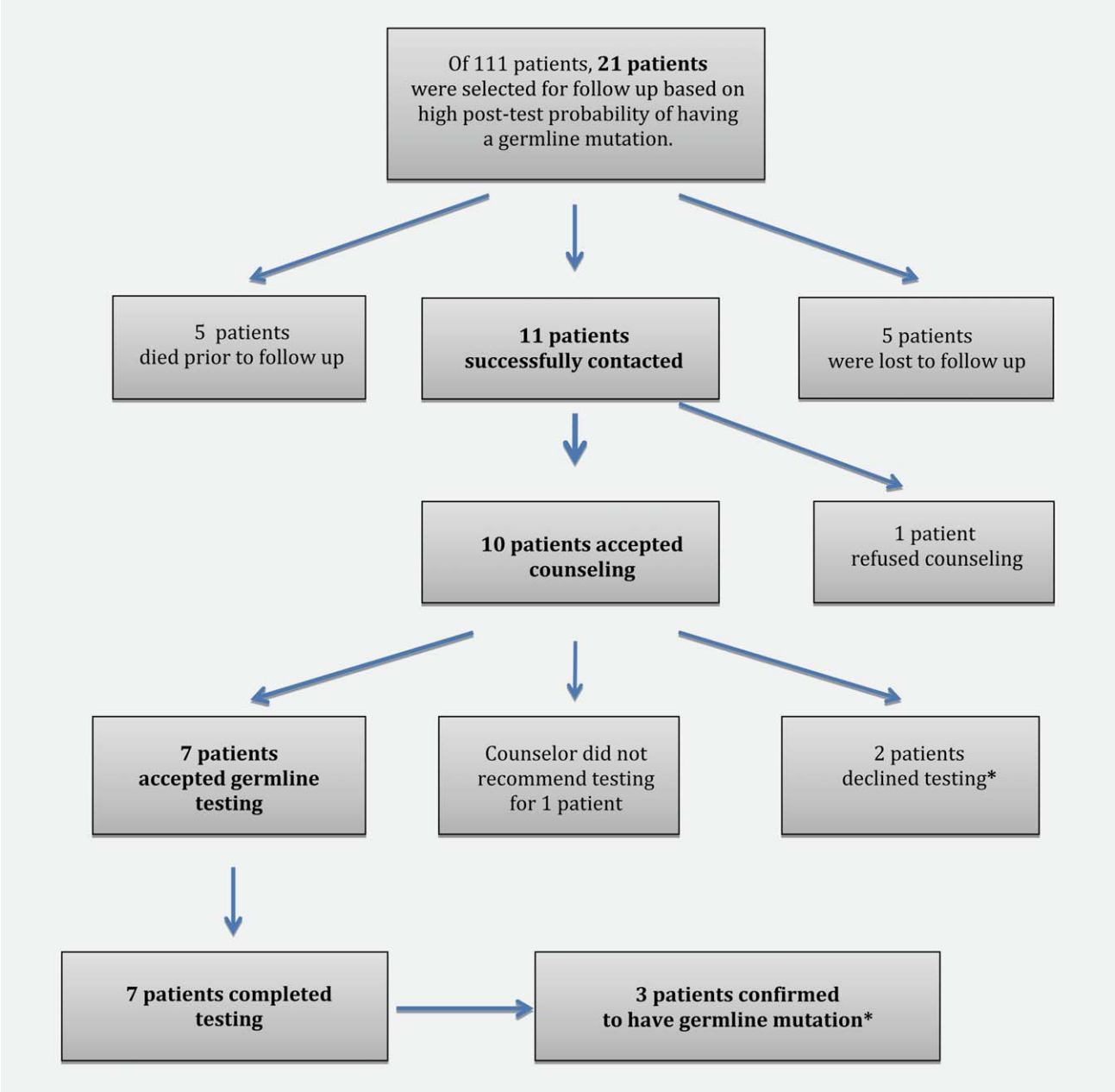

Figure 1. Outcomes of patients determined to have high post-test probability of carrying a germline mutation. *Patients were considered evaluable if they could be contacted and agreed to genetic counseling. Eight patients were considered evaluable, and three (37.5%) of these were confirmed to have a germline event (*all BRCA2*). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

panels were used as reference for selection of potential germline events from the tumor-only NGS results (Supporting Information). Those patients deemed to have a high likelihood of possessing a germline mutation in a known cancer risk gene, irrespective of family history, were identified and contacted for formal genetic counseling and germline sequencing. Only patients with clearly deleterious genomic events with known/suspected function were included. Genes with high frequencies of somatic alterations for which there are no actionable recommendations for individuals with germline mutations (*e.g.*, *CDKN2* and *TP53*) were excluded. Mutations in genes that are known to be frequently somatically mutated but also potentially germline (*e.g., CDH1* and diffuse gastric cancer) were further screened with family history, age and pathologic features to assign the post-NGS risk. After reviewing the NGS results and determining the post-NGS high risk patients, patients were considered evaluable if they could be contacted and if they agreed to genetic counseling.

Results

A total of 111 cases [64 (58%) gastroesophageal, 36 (32%) hepatobiliary, 10 (9%) colorectal, and 2 (2%) small bowel

Table 1. Identified high-risk patients contacted for counseling regarding potentially inherited cancer susceptibility syndromes after considering tumor NGS and clinical characteristics[1]

Case number	Tumor type	Gender	Age (years)	Family cancer history*	Tumor somatic aberrations	Germline findings
1	Gastric adenocarcinoma	F	29	Gastric (m. aunt) Breast (m. great aunt, m. great aunt) Prostate (father) Vaginal (p. grandmother)	***BRCA1 Y655fs*18***[2] *TP53 R196* ARID1A P279fs*117 CDH1* (introns not sequenced)	Not germline CDH1 (intron 4 VUS)
2	Cholangiocarcinoma	F	70	Breast (mother, m. aunt, m. aunt, m. grandmother) Lung (m. uncle) Thyroid (daughter)	***PTEN D52N,*** *GNAS R201H, TP53 R196,* CDKN2A/B loss CREBBP rearrangement TPM3-NTRK1 fusion FGF19 amp+ FGF4 amp+ FGF3 amp+CCND1 amp+*	Patient died prior to testing
3	EGJ	M	64	Pancreas (mother), Gastric, (m. grandmother)	***STK11 F219fs*53, BRCA2 H2417fs*4,*** *TP53 Y163C, ZNF217 amp+*	STK11 not germline (Peutz-Jeghers) (BRCA2 not germline)
4[3]	Cholangiocarcinoma/Colon	M	61	Breast (mother, sister), Bladder (brother)	Colon: ***BRCA2 K3326,* BRCA2 L2092fs*7,*** *APC Y1166,* TP53 R175H* Cholangio: ***BRCA2 K3326,* BRCA2 L2092fs*7,*** *KRAS G12D CDKN2A/B loss MCL1 amp+ MYC amp+ TP53 E51fs*1 GATA3 S382**	***BRCA2 K3326* L2092fs*7***
5	Gastric adenocarcinoma	M	67	Cholangiocarcinoma (sister) Lung (sister)	***BRCA2 T1345M BRCA2 S1982fs*22*** *KRAS G13D ERBB4 E1090D ARID1A V879fs*12*	***BRCA2 S1982fs*22 BRCA2 T1345M***
6	Gastric adenocarcinoma	M	81	Breast (mother) Ovary (mother) Lung (brother)	***MLH1 N168fs*34 MSH6 V592fs*6*** *PIK3CA H1047R PTCH1 R1308fs*64*	Patient died prior to testing
7	EGJ	M	77	Colon (sister)	***MLH1 S456**** *ERBB3 V104M NF1 R816* PIK3CA H1047Y ARID1A A1304fs*177 KDR R275* AXL T343M, CREBBP R1664C, CTCF N105fs*14*	Patient died prior to testing
8	Rectal adenocarcinoma	M	62	none	***BRCA2 S3366fs*4*** *KRAS G12V APC E991* E1397**	BRCA2 S3366fs*4
9	Gastric adenocarcinoma	F	76	Gastric? (mother, brother)	***CDH1 R74****	Not Germline
10	Gallbladder (cholangiocarcinoma)	M	25	Gallbladder "issues" in family	***ATM R1898**** *ARID1A S254fs*104*	Patient died prior to testing
11	Gallbladder/Cholangiocarcionma	M	76	Ovarian (p. cousin) colorectal (great aunt)	***STK11 loss CDKN2A/B loss*** *TP53 R337C*	Lost to follow up
12	Rectal adenocarcinoma	M	31	Colorecetal (m. grandmother) Lymphoma (m. great aunt x2).	***APC K1165**** *KRAS G12D (subclonal) ERBB4 amp+ TP53 I195T*	Accepted counseling; Refused germline testing
13	EGJ adenocarcinoma	M	38	none	***STK11 loss*** *AURKA amp+ CCND1 amp+ CDK6 amp+ KRAS amp+ TP53 R175H NFKBIA amp+ NKX2-1 amp+ FGF19 amp+ FGF4 amp+ ZNF217 amp+*	Patient died prior to testing
14	Gastric adenocarcinoma	F	43	ovarian (sister)	***CDH1 E518fs*4 CDH1 splice 1320+1G>T***	Not Germline
15	Gastric adenocarcinoma	F	47	Lung (father) colorectal (father)	***CDH1 F602fs*11*** *AKT1 amp+ TP53 R213**	Lost to follow up

Table 1. Identified high-risk patients contacted for counseling regarding potentially inherited cancer susceptibility syndromes after considering tumor NGS and clinical characteristics (Continued)

Case number	Tumor type	Gender	Age (years)	Family cancer history*	Tumor somatic aberrations	Germline findings
16	?gastric "unknown primary adenocarcinoma"	F	73	Bladder (p. grandfather)?breast (mother)	***BRCA2 K3326**** *BRAF V600E TP53 R306* SMAD2 R182* RNF43 S41* KDM5C E13fs*60*	Germline testing not recommended by genetic counselor as mutation was thought to be a germline polymorphism by Myriad Genetics
17	Gastric adenocarcinoma (also had colon cancer x 2—colon tumors not sequenced)	F	66	Colorectal (brother, father)	***MSH2 T44fs*20*** *ERBB2 V842I, ERBB3 G284R, TP53 R273C, TP53 Q144*, DNMT3A R882C*	Declined counseling and testing
18	Duodenal adenocarcinoma, Also has HCC for which genetic information was not obtained due to insufficient tissue	M	77	unknown	***MSH6 K1358fs*2***	Lost to follow up
19	Gastric adenocarcinoma	M	37	Lung, breast & colon (m. grandmother) lymphoma (p. grandmother)	***APC S1194*,*** *NF1 Q1841*-subclonal, TP53 I195T*	Accepted counseling; Refused germline testing
20	Gastric adenocarcinoma	M	70	EGJ cancer (personal hx) breast (mother) Breast (sister) Prostate (father) Gastric (p. grandfather)	***CDH1 W20fs*9***	Lost to follow up
21	Gastric adenocarcinoma	F	48	Brain (father)	***CDH1 splice site 531+1G>T***	Lost to follow up

*see Methods section for details.
[1]See supplementary files for more detailed history, and pedegrees of each case when available.
[2]Genes from somatic NGS that were considered potentially germline are bolded for each case.
[3]Cases bolded (case 4, case 5 and case 8) had confirmed germline mutation.
Abbreviations: VUS, variant of unknown significance; EGJ, esophagogastric junction; m. maternal; p. paternal.

Table 2. Distribution of post-NGS high-risk ($N = 21$) and confirmed germline ($N = 3$) cases by pre-NGS risk

Risk group based on Pre-NGS probability	Description of Pre-NGS groups	Post-NGS high-risk[1] cases: % of high risk patients post-NGS, % confirmed germline
High	• Strong family or personal history of malignancy, per current tumor-specific genetic counseling guidelines. • Ashkenazi Jewish heritage	Cases: (1,2,**5**,12,17,19,20): 33% (7/21), 14% (**1**/7)
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.	Cases: (3,**4**,6,11,14–16,21): 38% (8/21), 13% (**1**/8)
Low	• Unimpressive family history (either no known history of malignancy or remote, isolated cases)	Cases: (7,**8**,9,10,13,18): 29% (6/21), 17% (**1**/6)

[1]See methods regarding how post-NGS high risk was determined.
Bolded cases were confirmed to have germline events (see Table 1) (only 8 of 21 high risk patients were deemed evaluable for confirmatory germline testing—see methods and Fig. 1).
Abbreviation: NGS, next generation sequencing of tumor tissue.

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.

cancers] were analyzed *via* routine NGS of tumor samples. Twenty-one (19%) had mutations in one or more genes within the familial cancer gene panels, and were selected for follow-up based on high post-NGS probability of having a germline mutation (Fig. 1, Table 1). Fourteen (67%) of these 21 selected high post-NGS cases were gastroesophageal, 4 (19%) were hepatobiliary, 1 (5%) was small bowel and 3 (14%) were colorectal. One patient (Case 4) had 2 primary tumors (rectal adenocarcinoma, cholangiocarcinoma), for a total of 22 tumors analyzed in 21 patients. Detailed information regarding each case along with pedigrees, when available, is online (Supporting Clinical Information).

The 21 identified high-risk cases, determined after tumor-only NGS results (in other words, the patients with a high "post-NGS" risk), varied in their pre-NGS probability for an inherited cancer susceptibility syndrome (Tables 2 and 3). These patients were roughly grouped into three basic pre-NGS categories—high, intermediate and low (see Methods section, Table 3). Within the pre-NGS high-risk group were cases 1, 2, 5, 12, 17, 19, 20, accounting for 7 of 21 cases

(33%). The "intermediate-risk" pre-NGS group (the most common group) consisted of patients having some familial malignancies, but they did not fit neatly into traditional guidelines to necessarily prompt genetic testing, and included cases 3, 4, 6, 11, 14–16, 21 (8 of 21, 38%). A third group consisted of patients with an unimpressive family history who would be considered to have a low pre-NGS risk of an underlying hereditary syndrome; these included cases 7–10, 13, 18 (6 of 21, 29%).

In all pre-NGS risk categories, when tumor NGS did identify a mutation in a gene that is rarely somatically mutated (see Methods section, *e.g.*, *BRCA2*), investigators were led to suspect a hereditary syndrome that had not previously been anticipated. In the intermediate and low pre-NGS categories, these were the cases that would otherwise have gone unrecognized. Also, within the intermediate-risk group was a special case (case 4, Table 1) where DNA was sequenced from two separate primary tumors. Both tumors were found to have the same *BRCA2* mutation. In this setting, even absent confirmatory germline testing, this mutation was deemed likely germline given the extremely low probability of an identical somatic mutation occurring independently in two separate tumors.

Of the 8 evaluable high-risk patients post-NGS, 7 were deemed appropriate for confirmatory germline testing—the other was deemed to have a polymorphism in *BRCA2* and no germline testing was recommended. Of the 7 patients who ultimately underwent germline testing, 3 (37.5% of evaluable high-risk patients)—*one from each pre-NGS risk group*—were confirmed germline *BRCA2* carriers (Fig. 1, Table 2). Case 5 was considered high-risk pre-NGS due to Ashekenazi Jewish heritage, despite lack of strong family history of classic *BRCA2* tumors (breast/ovary). Case 4 was considered intermediate risk pre-NGS given the history of *potentially* two primary synchronous tumors (colon cancer and cholangiocarcinoma) in the patient, along with breast cancer in his mother and sister, and bladder cancer in his brother. Case 8 was considered low risk pre-NGS given rectal cancer without any other reported family history of cancer.

Discussion

Clinical oncology is in the midst of a major paradigm shift. Fueled by tremendous advances in molecular biology and technology, decisions regarding cancer care are increasingly being driven by data derived from NGS, which will exponentially increase as we continue the quest for "personalized medicine."[4] This change generates an array of ethical, legal and communications dilemmas related to the evaluation of genetic susceptibility to cancer, most of which do not have a clear solution or evidence-based guidelines. Technology continues to outpace our ability to assimilate and adapt.

High-throughput NGS is altering our traditional model of cancer genetics and genetic counseling. Current genetic counseling models are inadequate to address the unique nature of *germline* NGS,[5] let alone somatic tumor-only NGS. Patients are often "information-saturated" after 20–40 min of discussing a single gene or one hereditary cancer syndrome; it is even more challenging to address multiple diverse genes simultaneously.[5,7] The well-established screening and prevention guidelines for high-risk genes such as *BRCA1/2* required decades to develop.[5] Other genes included in current NGS platforms still have no consensus on the risks conferred nor do they have corresponding management guidelines.[5] However, a positive germline result, even of low/uncertain clinical impact, may motivate patients and their families to pursue healthy behaviors including risk reduction and preventative measures.[20–22]

Tumor-only NGS testing is now being performed *without* simultaneous germline testing, generally in the absence of pretest counseling. Tumor-only genome analysis adds yet another layer of complexity, due to the uncertain nature of any aberrations that are identified. Although the patient may understand that the test was performed to determine treatment or prognosis,[15] generally it is not until *after* a suspicious alteration is identified that they are informed that the somatic mutation *could* be an underlying germline mutation that may have further health implications for the patient and his or her family.

A patient's personal and family history of cancer may assist in gauging the degree of pretest counseling required prior to proceeding with tumor DNA profiling (Table 3). Raising pretest awareness that follow-up germline testing may be recommended to further evaluate somatic *versus* germline is important. The accuracy of patients' reporting family history, however, can range from 57–90% (depending upon the study method). Standardized tools for collecting family history to optimally direct appropriate patients to the genetic counselor, *prior to* obtaining NGS testing, may be useful.[23] However, it is not feasible for busy oncology clinics to implement detailed family risk screening for every patient, and innovative tools to electronically capture family history data in oncology practices are urgently needed.

Given our findings, it has become our practice to first assess family history and also discuss the implications of "tumor-only" sequencing *before* testing, as it pertains to the incidental identification of potential germline events, with particular emphasis in those with a significant family history of malignancy, Ashkenazi Jewish ancestry, or a previous personal history of cancer. A framework of pre- and post-test recommendations for the treating physician ordering tumor NGS is displayed in Table 3, where the degree of discussion is tailored based on the estimated pre-NGS risk. For high-risk patients, it should be emphasized that the results may strongly suggest an underlying inherited mutation. It is also strongly recommended that when NGS of synchronous (or metachronous) tumors is planned that the patient be counseled *prior* to ordering NGS on the second tumor, in case a mutation is found which would clearly point to a germline mutation even without confirmatory germline testing, such as in case 4.

Regarding the low pre-NGS risk group, these may still warrant a brief discussion of the rare possibility of identifying a potential germline event. This is exemplified by case 8, where there was no known family history of cancer. The

confirmation of a germline *BRCA2* mutation in this case raises many challenging questions. *BRCA2* somatic mutations have been rarely described (~2% of tumors COSMIC; 9% TCGA gastric cohort). To ascertain whether the majority of these "low risk" cases merely represent somatic mutations of otherwise well-characterized germline genes or true underlying germline events with either low penetrance, or a *de novo* mutation, requires further prospective investigation.

A limitation of this study is the retrospective nature and delayed counseling and sequencing of germline DNA, occuring only after post-NGS probability risk was estimated in patients—a reflection of the reality of current practice. Because of this, there were several identified high-risk patients post-NGS where germline testing was no longer possible (14/21, 67%) for various reasons (Fig. 1).

Of eight evaluable cases where germline testing was possible *and* in 7 cases recommended by the genetic counselor, three (37.5%) had confirmed germline mutations (all were *BRCA2*)—one case in each of the pretest risk categories (Table 2). Therefore, upon learning of a mutation from tumor-only NGS testing in a rarely somatically mutated gene, as in case 8, such a patient could be referred to the genetic counselor and evaluated for germline testing, irrespective of other risk factors. In contrast, genes that are frequently somatically mutated, such as *CDH1* in gastric tumors (up to 50% in some series), remain a low probability of being germline—as we observed in 2 of 2 *CDH1* cases which were able and recommended to undergo germline tested in our series (cases 9 and 14). Identifying patients at risk for hereditary syndromes entailing genes that are also notably frequently somatically mutated, such as *CDH1*, should follow established methods.[24]

Since tumor NGS sequencing will increasingly be performed within research and nonresearch settings,[4] and unexpected findings will continue to be uncovered, previously published guidelines and recommendations regarding appropriate testing[5,20] need to be revised and applied to somatic testing. Consent for somatic NGS testing currently only requires permission for tissue release from outside pathology departments. Contemporary counseling models were not designed for in-depth education about simultaneous testing of multiple genes or the unexpected discovery of *germline* mutations, not to mention somatic events that *could* be germline. Cancer centers, private practices and universities will have to develop and implement efficient mechanisms for pre-test counseling and informed consent prior to somatic-only testing, and disclosure mechanisms (full *vs.* selective *vs.* tiered) afterwards.[6,25] Whether to involve family members in the process, how to respond to moderate, high or uncertain penetrance results or multiple mutations, how to communicate definitions and terms surrounding genetic testing and differentiate germline from somatic testing will require new paradigms and a more in-depth informed consent than is currently practiced. Furthermore, tension between patient autonomy and confidentiality, and duty to warn family members of known genetic risks will occur,[26,27] including instances when, as in many of our cases, advanced cancer patients die prior to obtaining their genetic results. A written directive designating those who should be privy to that information may be appropriate.[28] Creating a repository of genetic information may be helpful for surviving family members as new information emerges on the significance of previous findings.[28]

In summary we retrospectively evaluated 111 patients that had undergone tumor-only NGS testing for treatment purposes. We identified 21 patients as "high risk" for underlying germline mutations based on the sequencing results. Because of the retrospective nature of the study, only 8 of the 21 patients were considered evaluable for genetic counseling and germline testing. However, of these 8 evaluable patients, we confirmed three germline mutations—interestingly evenly distributed across each of the three pre-NGS risk groups. This study highlights the potential of NGS tumor-only testing to detect underlying germline mutations in patients where a hereditary cancer susceptibility syndrome would not have been suspected and/or in patients that have pre-NGS high risk but have not yet received formal counseling regarding the implications prior to receiving the tumor-only NGS results. To formulate effective guidelines for screening and communicating somatic and consequent germline genetic information to patients, more investigation is required. Although NGS poses challenges for both germline and somatic DNA testing (and now circulating tumor DNA in plasma), we should embrace the technological advance and its potential to improve oncologic care on various fronts, while at the same time identifying areas requiring attention, so to generate guidelines on how to best proceed. Actively involving all stakeholders, including oncologists, bioethicists, genetic counselors, policy makers, insurance company representatives and the public will be vital. As we race closer towards individualized medicine, responsible and effective implementation of NGS technology is crucial so we are ready for surprises.

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