The Future of Parallel Tumor and Germline Genetic Testing: Is There a Role for All Patients With Cancer?

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ABSTRACT

Under the traditional paradigm of genetic testing in cancer, the role of germline testing was to assess for the inherited risk of cancer, whereas the role of tumor testing was to determine therapeutic selection. Parallel tumor-normal genetic testing uses simultaneous genetic testing of the tumor and normal tissue to identify mutations and allows their classification as either germline or somatic. The increasing adoption of parallel testing has revealed a greater number of germline findings in patients who otherwise would not have met clinical criteria for testing. This result has widespread implications for the screening and further testing of at-risk relatives and for gene discovery. It has also revealed the importance of germline testing in therapeutic actionability. Herein, we describe the pros and cons of tumor-only versus parallel tumor-normal testing and summarize the data on the prevalence of incidental actionable germline findings. Because germline testing in patients with cancer continues to expand, it is imperative that systems be in place for the proper interpretation, dissemination, and counseling for patients and at-risk relatives. We also review new therapeutic approvals with germline indications and highlight the increasing importance of germline testing in selecting therapies. Because recommendations for universal genetic testing are increasing in multiple cancer types and the number of approved therapies with germline indications is also increasing, a gradual transition toward parallel tumor-normal genetic testing in all patients with cancer is foreseeable.

J Natl Compr Canc Netw 2021;19(7):871–878
doi: 10.6004/jnccn.2021.7044

Historical Approaches to Genetic Testing in Cancer

Although the inherited component of cancer susceptibility has been appreciated for decades, novel gene discoveries and new implications for therapeutics have highlighted its importance in modern cancer care.¹ Traditionally, germline genetic testing involved a detailed review of personal and family cancer history with subsequent phenotype-driven genetic testing, often performed sequentially with 1 or 2 genes at a time. The primary purpose of germline genetic testing was to identify those with an inherited risk for cancer who could then benefit from tailored cancer screening/risk-reducing measures and cascade testing of at-risk family members.^{2,3} The decreasing costs of next-generation sequencing technologies and the widespread use of multigene panels have improved accessibility to testing and facilitated the discovery of novel gene–cancer associations and the incorporation of genes with varied cancer risk estimates from low to high penetrance.³ In addition, the increasing number of cancer susceptibility genes that simultaneously drive cancer risk and may direct cancer therapy, such as *BRCA1/2* mutations in hereditary breast and ovarian syndrome,⁴ has placed increasing importance on genetic testing in cancer care.¹ As a result, more patients with cancer are undergoing genetic testing, and there is a drive for universal germline testing for all patients with cancer.⁵

Similarly, in the era of precision medicine, the scope of tumor testing has also expanded, driven by the discovery of novel genotype-directed targeted therapies.^{6,7} Whereas tumor sequencing was traditionally performed in limited situations and often only in patients with advanced or metastatic cancer for genotype-directed clinical trial eligibility, it is now expanding into other clinical scenarios as treatment implications become more widespread.⁸ The recent NCI-MATCH study showed not only the feasibility of large-scale, pan-cancer tumor testing for the identification of clinically actionable mutations, but also the ability to assign patients to genotype-directed treatment in a clinical or research setting.⁹ Although germline genetic testing and tumor somatic testing were traditionally performed independently, novel

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parallel sequencing technologies have juxtaposed the 2 processes with widespread implications.¹⁰ Herein, we discuss the pros and cons of tumor-only versus parallel tumor-normal testing and review the implications and future of parallel testing.

Tumor-Only Versus Parallel Tumor-Normal Testing

The 2 approaches to the genomic testing of tumors include (1) the sequencing of tumor tissue only, wherein the tumor sequence analysis includes both somatic and germline alterations (tumor); and (2) the sequencing of tumor and matched normal tissue with the subsequent subtraction of germline alterations from somatic alterations, resulting in the identification of somatic mutations only on tumor assessment (somatic).¹¹ Notably, although germline sequencing occurs in *both* the tumor-only and the tumor-normal matched approach, in the former, the germline assessment is an *indirect* result of the tumor sequencing and in the tumor-normal analysis, the germline is *directly* interrogated. The parallel tumor-normal matched approach allows for a direct differentiation of somatic versus germline findings and the potential for the return of germline-specific genetic test results to the patient (Figure 1).^{10,12,13}

The advantage of a tumor-only approach is efficiency, because germline DNA is not directly interrogated, and the

necessary informed consents, privacy protections, and pre-testing/posttesting genetic counseling inherent to traditional germline genetic testing are not routinely needed.^{14,15} For the identification of therapeutically actionable genetic mutations, knowledge of the origin of the mutation as somatic versus germline is not always necessary. In addition, novel computational algorithms may eventually facilitate the accurate identification of somatic versus germline variants from tumor testing alone, raising the possibility of the return of germline results in the context of appropriate pretest informed consent.¹⁶

The disadvantages include challenges to the interpretation of results and the need for additional germline testing in certain patients, which could lead to delays in management and cascade testing. For example, tumor-only testing of a breast cancer sample may identify a *BRCA1/2* mutation, but it is unclear whether this mutation is somatic or germline. Additional genetic testing would be necessary but could potentially delay decisions regarding surgical management (mastectomy vs lumpectomy) and notification of at-risk relatives. Notably, negative tumor-only testing is not an adequate surrogate for dedicated germline testing, especially when the implications for cancer surveillance, treatment, and cascade testing are so critical. As recently shown by Lincoln et al,¹⁷ 8.1% of pathogenic germline mutations were missed in patients who previously underwent tumor-only sequencing

Figure 1. Pros and cons of tumor-only versus paired tumor-normal genetic testing. This figure depicts the results of germline, tumor, and paired tumor-normal sequencing and displays how paired sequencing allows the differentiation of germline (red) versus somatic (blue) results. Abbreviation: MSI, microsatellite instability.

because of the technical limitations of tumor sequencing, variant interpretation differences, or differences in the genes tested in the tumor versus the germline.

Perhaps most importantly, in tumor-only testing, the germline nature of findings may be misinterpreted by providers inexperienced in cancer genetics or incorrectly relayed to patients, resulting in suboptimal care not only for the patient but also for at-risk family members.¹⁸ As a result, the American College of Medical Genetics and Genomics¹⁹ and NCCN²⁰ have issued guidelines for the reporting of potential germline variants from tumor testing, and laboratories and clinicians should receive guidance and training on interpreting tumor-only testing and potential germline findings.^{21,22} Even in tumor-only testing, multidisciplinary care is often necessary to identify potential germline variants and facilitate genetic counseling and confirmatory germline testing.^{19,21–23}

The advantage of parallel tumor-normal testing is that it provides an unbiased assessment of the germline and somatic contribution to cancer risk and development.¹⁰ It also allows the integration of tumor and normal genomic data and complex interpretation, including the evaluation of loss of heterozygosity in tumor tissues and the differentiation of mosaicism and clonal hematopoiesis, which have implications for the heritability of findings.¹⁰ From a technical standpoint, it allows sample verification and additional quality control checks and may provide insight into the classification of germline variants.²⁴

Disadvantages include the extra costs, resources, and expertise that are necessary for the successful implementation, interpretation, and dissemination of results. There also need to be mechanisms in place for the timely and comprehensive management of any germline findings. In addition, the identification of “incidental” germline findings may be the double-edged sword of parallel tumor-normal testing. Although such germline findings may better elucidate cancer risk in families, the uncertainty of cancer risk estimates or gene penetrance in families without the corresponding phenotypic features make management decisions difficult, particularly in situations where risk-reducing surgeries may be relevant. For example, in a patient without a family history of gastric or lobular breast cancer, an incidental finding of a *CDH1* germline mutation on parallel testing makes decisions regarding prophylactic gastrectomy extremely difficult.^{25,26}

Parallel Testing Reveals Incidental Actionable Germline Findings

With the advent of tumor-normal testing, more clinically actionable germline findings are being discovered across cancer types, often in patients who do not meet traditional criteria for genetic testing.²⁷ In a study of 1,566 patients with paired tumor-normal testing using a 341-gene panel (MSK IMPACT),⁶ 246 patients (15.7%) had at

least 1 pathogenic or likely pathogenic variant in 1 of 187 Mendelian disease-associated genes.²⁸ A subsequent study examined 10,336 patients who underwent tumor-normal sequencing with MSK IMPACT, of which 1,040 were consented for germline analysis including 76 cancer-predisposing genes. Of these patients, 182 (17.5%) had clinically actionable variants conferring cancer susceptibility. Notably, 101, or 55% of germline-positive patients, would not have met the clinical criteria for guideline-based genetic testing.²⁹ Two smaller studies found that rates of discovery of pathogenic germline variants in patients with paired tumor-normal genetic testing were slightly lower, ranging from 2.3% to 4.3%.^{30,31} In the largest study, Huang et al³² used an automatic germline variant classification pipeline (CharGer) to analyze 10,389 patients who underwent tumor-normal testing from The Cancer Genome Atlas and found that 853 patients (8%) had pathogenic or likely pathogenic germline variants (Table 1).

New Opportunities for Gene Discovery and Novel Cancer Predisposition Syndromes

Parallel sequencing provides opportunities for the discovery of novel genes and predisposition syndromes in many new cancer types. In a study of 451 patients with locally advanced or metastatic prostate cancer who underwent parallel sequencing, germline findings were available in a subset of 221 patients, of whom 42 (19%) had pathogenic/likely pathogenic germline mutations in DNA damage repair genes.³³ In a study of 254 patients with renal cell carcinoma who underwent paired sequencing, 41 (16.1%) had germline mutations. Several of these patients (n=5) would not have met the clinical guidelines for genetic testing. In addition, an overrepresentation of *CHEK2* mutations was seen, suggesting a potentially novel association.³⁴ A similar study conducted in 586 patients with urothelial cancers, which are traditionally associated with mismatch repair deficiency (MMR-D) and Lynch syndrome, identified 54 individuals (9.2%) with pathogenic/likely pathogenic germline variants in DNA repair genes, excluding MMR genes.³⁵ Increasing repositories of patients with pan-cancers and paired tumor-normal testing have also allowed the discovery of novel cancer predisposition syndromes, particularly in individuals with multiple primary tumors.^{36,37}

Parallel testing also highlights the need for wider germline genetic testing in cancer types not traditionally associated with hereditary cancer syndromes. A study of tumor-normal sequencing in multiple samples from 88 patients with metastatic neuroendocrine tumors found that 14 patients (16%) had clinically actionable germline variants, suggesting a need for universal germline testing in this rare tumor type.³⁸ In a study of 15,045 patients who underwent paired testing to determine microsatellite

Table 1. Rates of Germline Findings in Patients Undergoing Parallel Tumor-Normal Testing

References	Study Design	Findings
Schrader et al ²⁸	1,566 patients with tumor-normal testing in a 341-gene panel (MSKCC IMPACT)—187 genes with Mendelian disease associations	246 patients (15.7%) had presumed pathogenic germline variants in known Mendelian disease-associated genes
Meric-Bernstam et al ³⁰	1,000 patients with tumor-normal testing of 202 genes	43 patients (4.3%) had likely pathogenic germline variants in 19 genes considered actionable; 2.3% of patients had previously unrecognized germline pathogenic mutations
Seifert et al ³¹	439 patients who underwent tumor-normal sequencing—36 hereditary cancer susceptibility genes	19 patients (4.3%) had germline variants indicative of hereditary cancer predisposition; for 10/19 patients, these findings were new diagnostic information
Mandelker et al ²⁹	10,336 patients with tumor-normal testing (MSKCC IMPACT)—1,040 patients with reported germline results from a panel of 76 genes	182 patients (17.5%) had clinically actionable mutations; 101 (9.7%) of these patients would not have met clinical criteria for germline testing
Huang et al ³²	10,389 patients who underwent tumor-normal testing; used automatic germline variant classification pipeline	853 patients (8%) had pathogenic or likely pathogenic germline variants

instability (MSI) status, Lynch syndrome was identified in 16.3% of MSI-high (MSI-H) tumors, 1.9% of MSI-indeterminate tumors, and 0.3% of microsatellite stable (MSS) tumors. Among the patients with Lynch syndrome, 50% had tumors other than classic colorectal or endometrial cancers, and 45% of these patients did not meet the clinical criteria for testing. This finding suggests that all patients with MSI-H or MMR-D tumors should undergo genetic evaluation for Lynch syndrome, and highlights the utility of integrating somatic and germline findings.³⁹ In addition, studies of patients with exocrine pancreatic cancer who underwent parallel tumor-normal genetic testing have found high rates of pathogenic germline alterations ranging from 15.1% to 19.8%.^{40,41} In fact, genomic alterations in exocrine pancreatic cancers are more likely to be germline than somatic,⁴⁰ and many are potentially clinically actionable.⁴² Accordingly, the recent NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic have recommended germline testing in all patients with pancreatic cancer.²⁰

Limitations of Parallel Testing

Although these studies are compelling and show that a large proportion of patients may benefit from universal tumor-normal genetic testing, there are limitations and challenges to parallel testing. Many of these studies were conducted at large, tertiary cancer centers and are subject to ascertainment bias, potentially inflating the rates of germline findings. In addition, it is unclear whether incidental germline findings in tumor types not traditionally associated with hereditary cancer syndromes are true drivers of disease or are merely passenger mutations. Indeed, especially common tumors such as lung, breast, prostate, and colorectal cancer can occur in individuals with germline mutations in cancer susceptibility genes in a sporadic fashion. Jonsson et al⁴³ examined *BRCA1/2* mutations

across pan-cancers and found that the role of these mutations in driving cancer phenotypes was dependent on tumor lineage and called into question the utility of clinical decisions based on these mutations. Latham et al³⁹ also found that a small proportion of patients with Lynch syndrome with germline mutations harbored tumors that were MSS and did not exhibit an MMR-D signature, suggesting that the germline mutations may be incidental. However, these studies also highlight the need to obtain both tumor and germline sequencing to fully understand the biology of tumors and separate the true drivers of disease from passenger mutations.

Further limiting the universal adoption of parallel testing are difficulties in the interpretation of “normal” tissue when sequencing blood due to clonal hematopoiesis (CH) and mosaicism, which can confound germline results. CH refers to somatic mutations in the hematopoietic lineage that can be identified through the sequencing of blood for normal tissue, and mosaicism refers to differential cell populations with distinct genotypes within one individual. Although genetic variants associated with CH or mosaicism should be present at lower variant fractions than the traditional 50% of germline heterozygous mutations, technical challenges in sequencing and early mosaicism events can make interpretation challenging.^{44,45}

In addition, many centers may not have an existing genetic counseling infrastructure to provide a comprehensive discussion of the risks/benefits of genetic testing and a timely dissemination of sensitive germline results and notification of at-risk relatives.⁴⁶ Results from the MAGENTA (Making GENetic Testing Accessible) study found that electronic genetic education and the release of results without traditional genetic counseling was noninferior with regard to patient distress and was associated with higher test completion rates, suggesting that a paradigm of individualized genetic counseling for patients

with positive findings alone may be acceptable.⁴⁷ There are also challenges related to informed consent, privacy protection, and future research regarding these germline specimens and data generated through parallel testing.^{48,49} Given these challenges, working groups have been formed to discuss best practices for the integration of somatic and germline data to facilitate the interpretation and application of data.²⁴

Germline Findings Can Lead to Novel Therapeutic Implications

Despite these limitations and challenges, the necessity of parallel tumor-normal testing is becoming more apparent as the actionability of germline findings expands with an increasing number of FDA-approved therapeutics with germline indications (Table 2). Early studies of targeted therapies for germline indications were conducted in basal cell carcinomas⁵⁰ and thyroid cancers.⁵¹ Both studies included a significant number of patients with known genetic cancer predisposition syndromes—Gorlin syndrome and multiple endocrine neoplasia 2 syndrome—and found efficacy in those populations. Recently, another *RET* inhibitor, selpercatinib, was approved for *RET*-mutant medullary thyroid cancers.⁵² Targeted therapies, including everolimus and selumetinib, have also been studied in rare, genetic conditions associated with multiple cancer types, such as tuberous sclerosis and neurofibromatosis 1.^{53–55}

Subsequently, a study of the PD-1 inhibitor pembrolizumab in patients with advanced/metastatic solid tumors with MMR-D who experienced disease

progression on traditional chemotherapy found efficacy in this population. The study included many patients with Lynch syndrome with tumors exhibiting MMR-D, the hallmark of Lynch syndrome-associated cancers.⁵⁶ As a result, pembrolizumab received the first tumor-agnostic indication from the FDA for all MMR-D/MSI-H tumors, including those in patients with Lynch syndrome.

More recently, PARP inhibitors have gained multiple germline-based FDA approvals in a variety of tumor types. In women with ovarian cancer and germline *BRCA* mutations, the PARP inhibitor olaparib was shown to be effective as a treatment in the recurrent setting and as maintenance in the up-front setting.^{57,58} Subsequently, multiple PARP inhibitors have been approved in ovarian cancer for a wider population of women, including those with somatic *BRCA* mutations,⁵⁹ those with homologous repair-deficient (HRD) tumors,^{60,61} and in all patients as maintenance therapy.⁶² PARP inhibitors are also approved in metastatic breast cancer for patients with germline *BRCA1/2* mutations.^{63,64} Interestingly, in metastatic prostate cancer, rucaparib is approved for patients with *BRCA1/2* mutations^{33,65}; however, olaparib is approved in those with a wider range of germline HRD genes.^{66,67} Olaparib was also recently approved in patients with metastatic pancreatic cancer and germline *BRCA1/2* mutations.⁴² These studies suggest that knowledge of germline mutations, particularly those in *BRCA1/2* or other HRD genes, may influence the effectiveness of PARP inhibitors and affect clinical management.

Table 2. Selected FDA Approvals for Germline Indications Across Cancer Types

Cancer Type	Drug	Germline Variant	Evidence
Breast cancer	Olaparib	<i>BRCA1/2</i>	OLYMPIAD (Robson et al ⁶⁴)
	Talazoparib	<i>BRCA1/2</i>	EMBRACA (Litton et al ⁶³)
Ovarian cancer	Olaparib	<i>BRCA1/2</i>	Study 42 (Domchek et al ⁶⁷) and SOLO1 (Moore et al ⁵⁸)
	Rucaparib	<i>BRCA1/2</i>	Study 10/ARIEL2 (Swisher et al ⁵⁹)
	Niraparib	<i>BRCA1/2</i>	QUADRA (Moore et al ⁶¹)
Metastatic prostate cancer	Olaparib	HR genes	PROfound (de Bono et al ⁶⁶)
	Rucaparib	<i>BRCA1/2</i>	TRITON2 (Abida et al ⁶⁵)
Pancreatic cancer	Olaparib	<i>BRCA1/2</i>	POLO (Golan et al ⁴²)
Advanced/Metastatic solid tumors	Pembrolizumab	MMR-D (Lynch syndrome)	KEYNOTE 016 (Le et al ⁵⁶)
Basal cell carcinoma	Vismodegib	<i>PTCH1</i> (Gorlin syndrome)	ClinicalTrials.gov identifier: NCT00833417 (Sekulic et al ⁵⁰)
Thyroid cancer	Vandetanib	<i>RET</i> (MEN2)	ZETA (Wells et al ⁵¹)
	Selpercatinib	<i>RET</i> (MEN2)	LIBRETTO (Wirth et al ⁵²)
Subependymal giant-cell astrocytoma	Everolimus	<i>TSC1/2</i> (tuberous sclerosis)	EXIST-1 (Krueger et al ⁵⁵)
Renal angiomyolipoma	Everolimus	<i>TSC1/2</i> (tuberous sclerosis)	EXIST-2 (Bissler et al ⁵³)
Plexiform neurofibromas	Selumetinib	<i>NF1</i> (neurofibromatosis type 1)	ClinicalTrials.gov identifier: NCT01362803 (Dombi et al ⁵⁴)

Abbreviations: HR, homologous repair; MEN, multiple endocrine neoplasia; MMR-D, mismatch repair-deficient.

Future of Parallel Testing: Informing Treatment Decisions

As the number of germline indications for therapies—both FDA-approved and for clinical trials—increases, germline results from parallel tumor-normal testing are directly impacting treatment decisions.⁶⁸ In a study of 11,947 patients who underwent parallel tumor-normal testing with MSK IMPACT, 1,042 (9%) had a likely pathogenic/pathogenic germline alteration in a gene with a targeted therapy that was FDA-approved or had supporting data of such quality that it might warrant future approval. Most mutations were in *BRCA1/2*, *ATM*, or Lynch genes, and the most common cancer types represented were ovarian, breast, pancreatic, and prostate cancers. Notably, among the subset of patients with advanced cancer, 40% received germline-directed treatment. Common targeted therapies included PARP and checkpoint inhibitors.⁶⁹ These studies highlight the importance of parallel testing in expanding the population of patients receiving germline genetic information to not only improve the assessment of inherited risk but also expand therapeutic options.

Expanding Germline Testing to All Cancer Types?

Given the increasing therapeutic implications of germline findings, there have been major updates to recommendations for genetic testing in various cancer types.⁷⁰ Although universal germline testing for *BRCA* mutations had been recommended for ovarian cancer, this recommendation has now expanded to patients with metastatic breast cancer, metastatic prostate cancer, and pancreatic cancer. In addition, there are expanded recommendations for MMR staining and MSI testing in all patients with colorectal and endometrial cancer. As germline testing expands across tumor types, there is a question of whether patients with earlier-stage, nonmetastatic cancers should undergo germline testing because targeted therapies are moving into earlier stages of disease. For example, there are ongoing adjuvant studies of checkpoint inhibitors in patients with Lynch syndrome with endometrial cancer (GY020; ClinicalTrials.gov identifier: NCT04214067)

and colon cancer (NCT02912559) and adjuvant studies of PARPi in women with breast cancer and germline *BRCA* mutations (OlympiA; NCT02032823). Preliminary data also support the efficacy of PARPi monotherapy as neoadjuvant treatment in women with germline *BRCA* mutations and operable breast cancer,⁷¹ with an ongoing phase III trial (NCT03499353).

Call to Action: Parallel Testing in All Patients With Cancer

The integration of somatic and germline testing is critical to comprehensively characterize pathogenic germline mutations, accurately assess drivers of cancer risk, and make therapeutic decisions. Going forward, there may be a benefit to parallel next-generation sequencing tumor-normal testing for all patients with cancer. In addition, studies show that patients are very interested in paired testing and in obtaining germline results.⁷² As parallel testing becomes more common, there will be a need to carefully interpret results for both therapeutic potential and cancer predisposition risk. Challenges for moving forward include decisions on multigene, tumor-agnostic germline panels versus more disease-directed panels, and the need for innovative systems to provide the appropriate counseling and dissemination of results. However, expanded germline testing may continue to have unforeseen benefits, such as expanded opportunities for cascade testing,⁷³ potentially benefiting not only patients but also their at-risk relatives.

Submitted September 13, 2020; final revision received December 28, 2020; accepted for publication April 9, 2021.

Disclosures: Dr. Liu has disclosed receiving grant/research support from AstraZeneca and GlaxoSmithKline/Tesaro. Dr. Stadler has disclosed having an immediate family member who serves as a consultant in ophthalmology for Alcon, Adverum Biotechnologies, Allergan, Genentech/Roche, Novartis, Neurogene, Gyroscope Therapeutics, Optos, Regeneron, and RegenxBio.

Funding: This work was supported by funding from the NIH (MSKCC is supported by the NCI under Core Grant P30 CA008748).

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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