Rate of incidental germline findings detected by tumor-normal matched sequencing in cancer types lacking hereditary cancer testing guidelines

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INTRODUCTION

Recent studies reveal the prevalence of pathogenic/ likely pathogenic (P/LP) germline variants in advanced cancer patients is greater than previously anticipated. Clinically actionable germline findings have been found in 13-18% of cancer patients but strikingly, over half would have been missed with a 2018 or 2020 NCCN guidelines-based approach (PMID: 33126242, 28873162).

Tumor-normal (T/N) matched next-generation sequencing (NGS) has multiple advantages including enhanced variant classification and incidental germline variant discovery that may be missed with a guidelines-based approach.

Here, we investigate the benefits of T/N NGS in detecting P/LP germline variants and P somatic variants in a real-world dataset comprised of cancer types lacking hereditary cancer testing guidelines.

METHODS

Tempus|xT Tissue Assay

This targeted 648 gene NGS panel is a laboratory developed test (LDT) that detects single nucleotide variants (SNVs), indels, and copy number variants (CNVs), as well as chromosomal rearrangements in 22 genes with high sensitivity and specificity (Beaubier et al., Oncotarget 2019). Incidental germline findings were limited to SNVs and small insertions/deletions.

Cohort Selection

We retrospectively analyzed next-generation sequencing data from de-identified records of 21,395 patients tested with Tempus|xT T/N matched LDT assay (Tempus, Chicago, IL), using formalin-fixed paraffin embedded tumor tissue and normal matched specimens (blood or saliva).

Real-world Evidence Analysis

Detection of P/LP incidental germline findings (i.e., SNVs and small insertions/deletions) in 50 hereditary cancer genes was queried in the following cohorts: bladder (n = 920), brain (n = 1,505) cholangiocarcinoma (n = 535), esophageal (n = 471), head & neck (n = 822) and lung (n = 6,022).

For comparison, we included 4 cancer types that frequently undergo germline testing: breast (n = 3,771), ovarian (n = 2,225), pancreatic (n = 2,758) and prostate (n = 2,366).

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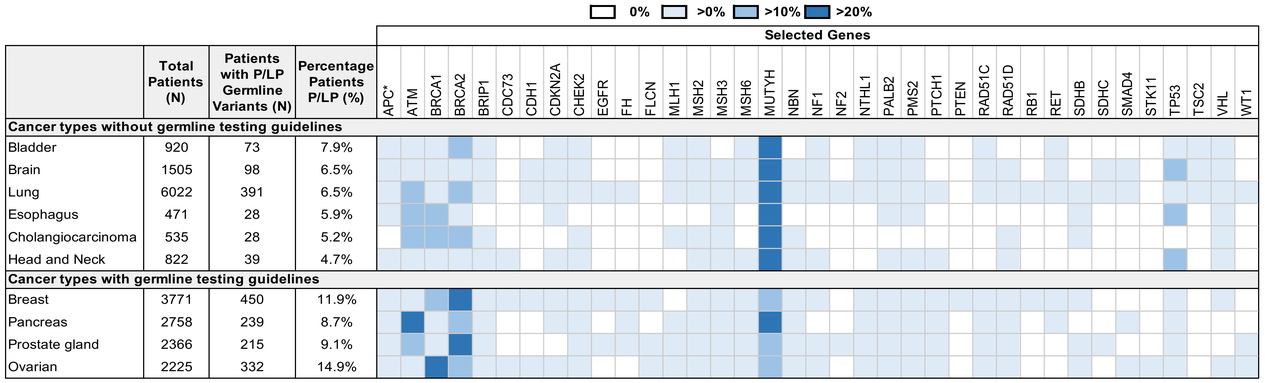
RESULTS

Table 1. Clinical Characteristics

	Total Patients, N	P/LP Germline Variants, N (%)
Cancer types without germline testing guidelines		
Overall	10,275	657 (6.4%)
Age at Diagnosis		
<=10	40	2 (5.0%)
11-20	58	5 (8.6%)
21-30	165	13 (7.9%)
31-40	307	29 (9.4%)
41-50	685	46 (6.7%)
51-60	1,978	122 (6.2%)
61-70	3,095	182 (5.9%)
71-80	2,467	172 (7.0%)
81-90	730	34 (4.7%)
>90	40	2 (5.0%)
Unknown	710	50 (7.0%)
Cancer types with germline testing guidelines		
Overall	11,120	1236 (11.1%)
Age at Diagnosis		
<=10	1	1 (100%)
11-20	10	1 (10%)
21-30	124	20 (16%)
31-40	595	91 (15%)
41-50	1,386	223 (16%)
51-60	2,642	307 (12%)
61-70	3,121	308 (9.9%)
71-80	1,882	154 (8.2%)
81-90	405	31 (7.7%)
>90	22	1 (4.5%)
Unknown	932	99 (11%)

Table 1. Total number of patients and patients with P/LP germline variants at age of diagnosis. Cancer types without germline testing guidelines include bladder, brain, cholangiocarcinoma, esophagus, lung, and head & neck. Cancer types with germline testing guidelines include breast, ovarian, pancreas, and prostate.

Figure 1. Prevalence of P/LP Germline Variants Detected by T/N Matched Sequencing Across Cancer Subtypes

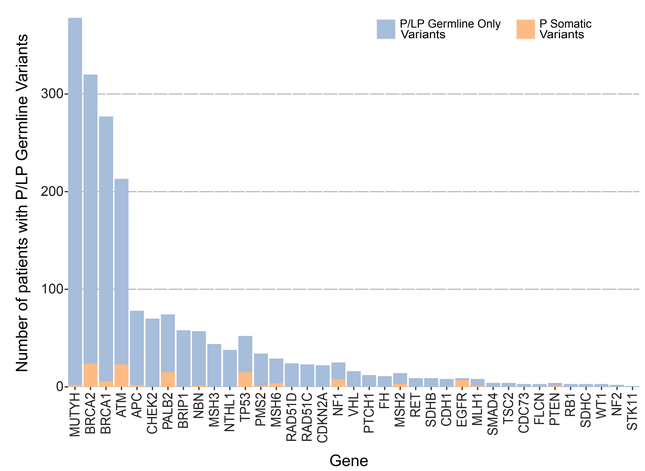


	Total Patients (N)	Patients with P/LP Germline Variants (N)	Percentage Patients P/LP (%)
Cancer types without germline testing guidelines			
Bladder	920	73	7.9%
Brain	1505	98	6.5%
Lung	6022	391	6.5%
Esophagus	471	28	5.9%
Cholangiocarcinoma	535	28	5.2%
Head and Neck	822	39	4.7%
Cancer types with germline testing guidelines			
Breast	3771	450	11.9%
Pancreas	2758	239	8.7%
Prostate gland	2366	215	9.1%
Ovarian	2225	332	14.9%

Figure 1. The heat map represents % of patients with germline variants in selected genes out of the total patients with P/LP germline variants by cancer subtypes. Genes without variants across all cancer types were excluded. Patients with at least one P/LP germline variant in *MUTYH*, *MSH3*, *and NTHL1* were included in the analysis. *ATM* and *BRCA2* were the two most prevalent genes with clinically actionable germline variants detected in lung and bladder cancer. *Ashkenazi Jewish founder variant p.I1307K was the most frequent mutation in *APC*.

Figure 2. Patients with "Second" Somatic hits

A



B

Gene	P Somatic Variants / Total P/LP Germline Variants (%)
APC	**2/78 (3%)**
ATM	**23/213 (11%)**
BRCA1	**6/277 (2%)**
BRCA2	**24/320 (8%)**
BRIP1	0/58 (0%)
CDC73	0/3 (0%)
CDH1	0/8 (0%)
CDKN2A	0/22 (0%)
CHEK2	0/70 (0%)
EGFR	**7/9 (78%)**
FH	0/11 (0%)
FLCN	0/3 (0%)
MLH1	**1/8 (12%)**
MSH2	**3/14 (21%)**
MSH3	0/44 (0%)
MSH6	**4/29 (14%)**
MUTYH	**2/378 (1%)**
NBN	**1/57 (2%)**
NF1	**8/25 (32%)**

Gene	P Somatic Variants / Total P/LP Germline Variants (%)
NF2	0/2 (0%)
NTHL1	0/38 (0%)
PALB2	**15/74 (20%)**
PMS2	**1/34 (3%)**
PTCH1	0/12 (0%)
PTEN	**1/4 (25%)**
RAD51C	0/23 (0%)
RAD51D	0/24 (0%)
RB1	0/3 (0%)
RET	0/9 (0%)
SDHB	0/9 (0%)
SDHC	0/3 (0%)
SMAD4	0/4 (0%)
STK11	0/1 (0%)
TP53	**15/52 (29%)**
TSC2	0/4 (0%)
VHL	0/16 (0%)
WT1	0/3 (0%)

Figure 2. A) The number of patients with a P/LP germline variant and a P somatic variant in the same gene across all cancer types. Note, 7/9 patients with an *EGFR* germline variant also had a P somatic hit. Patients with at least one P/LP germline variant in *MUTYH*, *MSH3*, and *NTHL1* were included in the analysis. **B)** Percentage of patients with a P somatic variant and a P/LP germline variant in the same gene. Note, 15/52 (29%) of the patients had a "second" hit in *TP53*, and 8/25 (32%) in *NF1*. Loss of heterozygosity (LOH) was not included in this analysis.

SUMMARY

We present the **largest retrospective analysis** to our knowledge on **de-identified real-world data** from patients diagnosed with **advanced cancer** with T/N matched sequencing data and present the prevalence of incidental P/LP germline variants in **cancer types lacking hereditary cancer testing guidelines**.

T/N matched sequencing may identify **incidental germline variants** missed by a guidelines-based approach to testing. The highest prevalence of incidental P/LP germline variants was identified in patients with **bladder (7.9%), brain (6.5%), and lung (6.5%) cancers**. The identification of such findings may have **clinical implications** for the patient and at-risk family members, resulting in the opportunity for **genetic counseling and risk-stratified intervention**.

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