Germline Findings From Tumor-Only Comprehensive Genomic Profiling in the RATIONAL Study: A Missed Opportunity?

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ABSTRACT

PURPOSE Tumor comprehensive genomic profiling (CGP) may detect potential germline pathogenic/likely pathogenic (P/LP) alterations as secondary findings. We analyzed the frequency of potentially germline variants and large rearrangements (LRs) in the RATIONAL study, an Italian multicenter, observational clinical trial that collects next-generation sequencing–based tumor profiling data, and evaluated how these findings were managed by the enrolling centers.

PATIENTS AND METHODS Patients prospectively enrolled in the pathway-B of the RATIONAL study and undergoing CGP with the FoundationOne CDx assays were included in the analysis. Potentially germline variants detected in 40 cancer susceptibility genes (CSGs) were classified in three classes with different actionability, most (MA), high (HA), and standard (SA), on the basis of penetrance, mutational spectrum, and intervention for prevention/early detection.

RESULTS On the basis of the European Society of Medical Oncology recommendations, we identified 225 potentially germline P/LP variants in 193/1,339 (14.4%) enrolled patients. In particular, 62/225 (27.5%) variants were detected in genes classified as MA-CSG class, 53/225 (23.6%) in genes belonging to the HA-CSG class, and 110/225 (48.9%) in the SA-CSG class. In addition, we detected 58 LRs in the 16/40 CSGs in 53/1,339 (3.95%) patients. Information about germline-focused analysis and follow-up was available for 99 patients with potentially germline variants. Surprisingly, 95/99 (96%) patients were not referred to oncogenetic consultation and follow-up, including 30/32 (93.75%) patients with variants in the MA-CSG class.

CONCLUSION Our data confirm the utility of CGP for the identification of potentially germline variants in CSGs, highlighting the importance of reporting LRs in addition to single-nucleotide variants and insertions/deletions. However, our findings also demonstrate a relative lack of knowledge of the implications of germline findings detected on tumor-only sequencing among oncologists and underline the need for specific training in this area.

ACCOMPANYING CONTENT

Data Supplement

Visual Abstract

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INTRODUCTION

Tumor comprehensive genomic profiling (CGP) with panels that allow for a broad molecular characterization, including the detection of mutational signatures and complex biomarkers (ie, tumor mutational burden [TMB], microsatellite instability, etc), can reveal actionable genomic alterations (GAs) for targeted therapeutic intervention in patients with solid tumors.[1-6] Although CGP has been implemented to identify somatic variants in tumor tissue or plasma samples, it may also detect potential germline pathogenic/likely pathogenic (P/LP) alterations as secondary findings.[5,7,8] The identification of germline alterations associated with increased cancer risk might have important clinical implications for patients and their relatives, in terms of cancer prevention and long-term follow-up.[1,9,10]

As CGP on tumor-only and plasma-only samples is not able to discriminate somatic from germline alterations, a confirmatory germline test should be performed for presumed

CONTEXT

Key Objective

To analyze the frequency of potential germline variants and large rearrangements (LRs) in cancer susceptibility genes (CSGs) in Italian patients with cancer undergoing tumor- or plasma-only sequencing with a validated next-generation sequencing test, and to evaluate the management of this information by the oncologists.

Knowledge Generated

A significant fraction of patients with advanced cancer carried potentially germline variants and LRs in CSGs. Genomic alterations in CSGs were detected also in tumors that do not usually undergo genetic surveillance, such as cancer of unknown primary, cholangiocarcinoma, and lung cancer. However, only 4% of patients carrying variants in CSGs were referred to genetic counseling.

Relevance

Our findings support the testing of CSGs in patients with cancer, to identify carriers in which health surveillance programs and interventions can lead to early diagnosis and prevention. Our data also highlight the need for specific training of oncologists, to ensure the correct interpretation of complex genomic data.

germline variants.[9] The European Society of Medical Oncology (ESMO) developed recommendations for confirmatory germline analysis and follow-up of tumor-only CGP.[11,12] The most recent recommendations suggest germline follow-up for single-nucleotide variants (SNVs) and small insertions/deletions (indels) identified in 40 cancer susceptibility genes (CSGs),[11] categorized into three classes on the basis of evidence of risk (Data Supplement, Table S1).

We analyzed the frequency of potential germline P/LP alterations detected in the RATIONAL study, an Italian multicenter, observational clinical trial that collects next-generation sequencing (NGS)–based tumor profiling data from patients with more of 40 tumor types. In addition, we evaluated how the discovery of possible germline variants was managed by the centers participating in the study.

PATIENTS AND METHODS

The RATIONAL study (ClinicalTrials.gov identifier: NCT05918666) is a multicentric, observational study approved by the Ethics Committee of the Istituto Nazionale Tumori Fondazione G. Pascale (Prot. 6/17 OSS) and by the ethics committees of other 48 participating institutions. All patients provided a specific written informed consent to participate to the study. Patients are informed about all GAs, including those that could affect family risk, unless patients opt out.

Between October 2018 and July 2024, 1,986 patients were enrolled, 390 in pathway-A and 1,596 in pathway-B. In pathway-A, patients with advanced solid tumor who already received a NGS-based tumor profiling are registered. In pathway-B, patients for whom no access to NGS was available and with specific clinical and pathologic features are offered access to the FoundationOne CDx assays (Foundation Medicine Inc, Cambridge, MA) on either tumor tissue or blood samples. Additional information is provided in the Data Supplement.

RESULTS

Patients

Between October 2018 and July 2024, FoundationOne CDx reports were successfully released for 1,341 patients enrolled in pathway-B. Two patients did not meet the inclusion criteria (age <18 years) and they were excluded from further analyses. Therefore, 1,339 patients were included in the study.

The clinical and pathologic characteristics of the whole cohort are described in Table 1.

CGP was performed on tissue with the FoundationOne CDx assay for 944 (70.5%) patients and on circulating tumor DNA (ctDNA) with the FoundationOne Liquid CDx test for 395 (29.5%) patients (Fig 1A).

Identification of Potentially Germline P/LP Variants by Tumor-Only CGP

Sequencing analysis revealed the presence of at least one GA with known significance in 1,297/1,339 (96.9%) patients. Overall, 6,807 GAs in 291 genes were identified, with a mean of 5.25 GAs per patient.

We evaluated the frequency of potentially germline P/LP GAs in the 40 CSGs of the ESMO recommendations, including SNVs, indels, and large rearrangements (LRs). We next applied the suggested variant allelic frequency (VAF) thresholds (VAF > 30% for SNVs and >20% for indels).[11] By

TABLE 1. Clinicopathologic Characteristics of Patients Enrolled in the RATIONAL Study Included in the Analysis

Characteristic	All (N = 1,339)
Age, years, median (range)	62 (18-87)
Age <30 years, patients, No. (%)	26 (1.94)
Sex, No. (%)	
Female	624 (46.6)
Male	715 (53.4)
Ancestry, %	
Caucasian	>99
Family history of cancer, No. (%)	
Yes	262 (19.57)
No	389 (29.05)
NA	688 (51.38)
Tumor type, No. (%)	
Lung cancer	342 (25.54)
Biliary tract cancer	271 (20.24)
Pancreatic cancer	165 (12.32)
Colorectal cancer	112 (8.36)
Esophagogastric cancer	107 (7.99)
CUP	85 (6.35)
Breast cancer	54 (4.03)
Soft tissue cancer	35 (2.61)
Head and neck cancer	34 (2.54)
Ovarian cancer	15 (1.12)
Renal cancer	15 (1.12)
Small bowel cancer	12 (0.90)
Uterine cancer	12 (0.90)
Bladder cancer	12 (0.90)
CNS cancer	11 (0.82)
Liver cancer	10 (0.75)
Thymus cancer	9 (0.67)
Pleural mesothelioma	8 (0.60)
Melanoma	8 (0.60)
Thyroid cancer	6 (0.45)
Skin cancer	5 (0.37)
Prostate cancer	3 (0.22)
Adrenal cancer	2 (0.15)
Carcinoma of the vulva/vagina	2 (0.15)
Penile cancer	2 (0.15)
Anal cancer	1 (0.07)
Testicular cancer	1 (0.07)

Abbreviations: CUP, cancer of unknown primary; NA, not applicable.

applying these criteria, one or more potentially germline P/LP variants above the VAF threshold were observed in 193/1,339 (14.4%) patients, including 23/193 patients (11.9%) with at least two potentially germline variants (Fig 1A). Collectively, we classified 225 variants in 30 genes (124 SNVs and 101 indels) as potentially germline. The most common genes in which potentially germline variants were observed pan-cancer were ATM (15.1% of potentially germline variants), MUTYH (10.7%), NF1 (10.7%), BAP1 (9.6%), BRCA1 (9.6%), BRCA2 (6.7%), and CHEK2 (5.3%; Fig 1B). For genes with more than 10 potentially germline mutations, we verified whether variants clustered in a specific region. For BRCA1, BRCA2, ATM, NF1, and BAP1, the variants were scattered over the multiple exons (data not shown). By contrast, for MUTYH and CHEK2, the variants clustered in specific hotspot regions (Data Supplement, Fig S1).

Potentially germline variants were mostly detected in lung (22.3% of patients carrying potentially germline variants), biliary tract (21.8% of patients), pancreatic (13% of patients), gastric (8.8%), breast (7.3%), and colorectal cancers (6.7%; Fig 1C). However, this distribution was largely influenced by differences in the number of patients with different histology enrolled in this study. In fact, the fraction of patients carrying potential germline mutations was higher in ovarian, breast, gastric, and pancreatic cancers, followed by biliary tract, colorectal, and lung cancers (Data Supplement, Fig S2 and Table S2).

The cohort of patients with potentially germline variants was younger than patients without potentially germline variants (P = .0033; Table 2). Males were more numerous than females, although the difference was not statistically significant. Median TMB was significantly higher in patients with potentially germline variants compared with patients without potentially germline variants (P = .0001). No statistical differences were observed between the cohort of patients with or without germline variants in terms of familial history or cancer, although the information was available for only 50% of patients (Table 2).

The majority of potentially germline mutations was detected by tissue testing (154/193; 79.8%), compared with ctDNA testing (39/193; 20.2%).

Classification of Potentially Germline Variants Into CSG Classes

We categorized the 225 potentially germline variants into CSG classes, accordingly with the ESMO classification[11] (Data Supplement, Table S1). Collectively, 62/225 (27.5%) variants were detected in genes classified as most actionable (MA)-CSG class, 53/225 (23.6%) in genes belonging to the high actionable (HA)-CSG class, and 110/225 (48.9%) in the standard actionable (SA)-CSG class.

Sixty-two potentially germline mutations (26 SNVs and 36 indels) were detected in seven MA-CSGs in 59 patients (Fig 2A). Potentially germline variants were most frequently detected in *BRCA1* (22/62 mutations, 35.5%), *BRCA2* (15/62 mutations, 24.2%), and *MSH6* (8/62 mutations, 12.9%; Fig 2A). The higher number of potentially germline variants belonging to the MA-CSG class was detected in non–small cell lung cancer (11/59, 18.6% of patients with MA-CSG class variants), breast cancer (10/59, 16.9%) and biliary tract cancer (9/59, 15.2%; Fig 2A and Data Supplement, Figs S3A

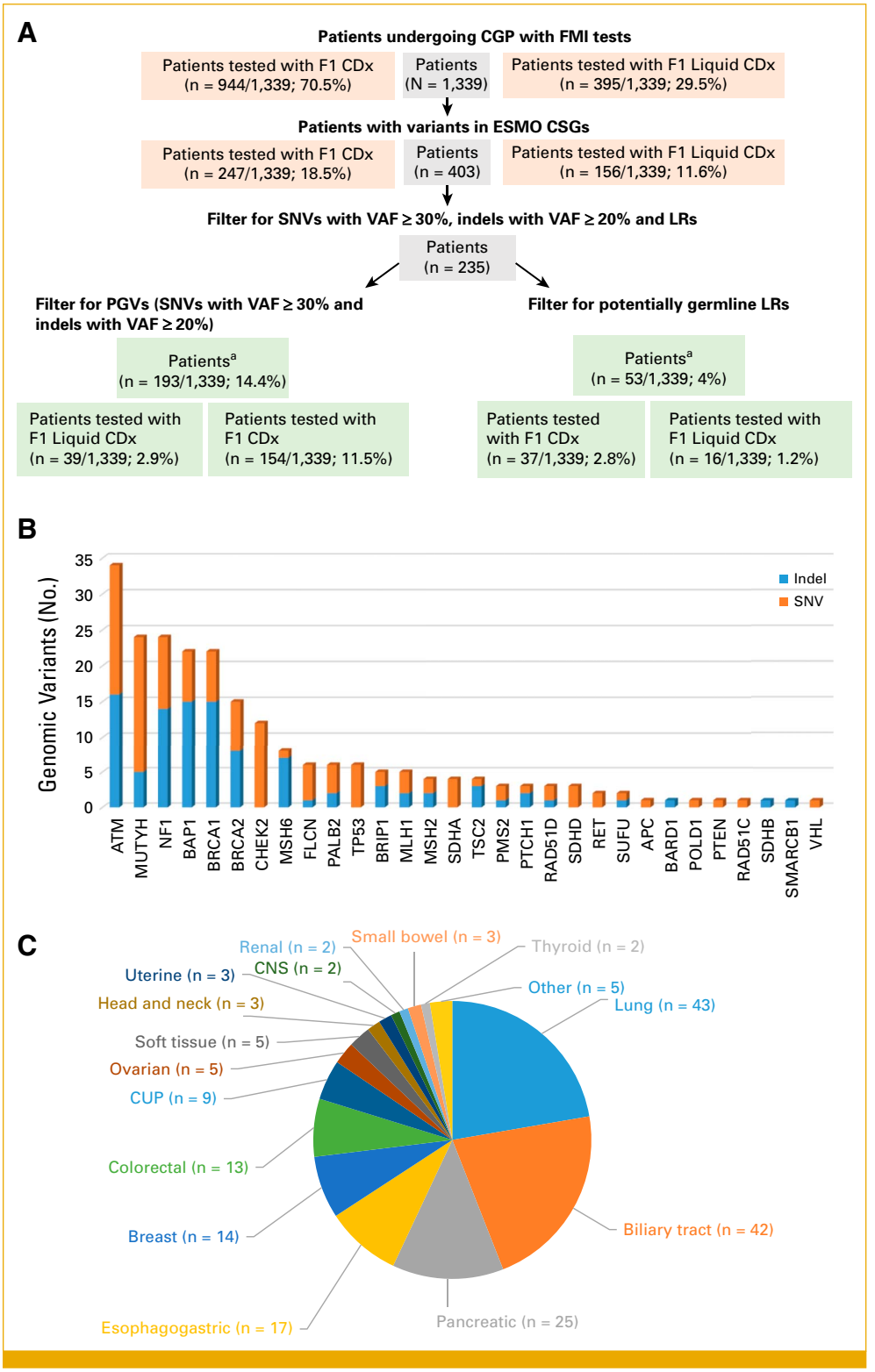

FIG 1. PGVs in the cohort of patients enrolled in the RATIONAL study. (A) Flowchart of the study. A total of 1,339 patients from the RATIONAL study were included in the analysis. Filters on the basis of the ESMO recommendations applied for identifying patients with potentially germline genomic alterations were shown. The percentage and the number of patients at each filtering step were indicated. [a]Eleven patients had both potentially germline PGVs and LRs. (B) Two hundred and twenty-five potentially germline genomic variants were identified in 40 CSGs recommended by ESMO for germline analysis and follow-up. (C) Distribution of patients (n = 193) with potentially germline variants accordingly to tumor types. Other: anal cancer (n = 1 patient); adrenal cancer (n = 1 patient); liver cancer (n = 1 patient); melanoma (n = 1 patient); and skin cancer (n = 1 patient). (continued on following page)

FIG 1. (Continued). CGP, comprehensive genomic profiling; CSG, cancer susceptibility gene; ESMO, European Society of Medical Oncology; FMI, Foundation Medicine; LR, large rearrangement; P/LP, pathogenic/likely pathogenic; PGV, potentially germline P/LP variant; SNV, single-nucleotide variant; VAF, variant allelic frequency.

and S3B). However, breast cancer was the tumor with the highest probability to detect MA-CSG class variants, that were found in 10/54 (18.5%) patients with breast cancer (Data Supplement, Figs S3A and S3B).

Familial history of cancer was reported for 12/59 (20.3%) patients, including four patients with breast cancer, three patients with pancreatic cancer, three patients with ovarian cancer, one patient with gastric cancer, and one patient with cholangiocarcinoma. For 21/59 (35.6%) patients, no familial history of tumor was reported. For 26/59 (44.1%) patients, the information was not available (Data Supplement, Table S3).

We classified 45 potentially germline mutations (39 SNVs and 14 indels) in nine genes in the HA-CSG class for which germline analysis and follow-up is suggested independently from age, and additional eight SNVs in three genes with follow-up recommended only for patients with tumors arising at age <30 years (Fig 2B). Overall, the 53 potentially germline variants were identified in 48 patients, including six patients with age <30 years. The majority of potentially germline mutations were observed in the *MUTYH* gene (24/53 variants; 45.3%) in 23 patients (Fig 2B). *MUTYH* variants were the only mutation in CSGs in 21 patients, whereas two patients carried also a *BRCA1* and a *PALB2* variant, respectively. Genomic variants were more frequently observed in pancreatic cancer (14/48, 29.2% of patients with HA-CSG class variants; 14/165, 8.5% of patients with pancreatic cancer), followed by colorectal and lung cancers (Fig 2B and Data Supplement, Figs S3A and S3C). Variants in genes belonging to the HA-CSG class were also detected in different tumors at low frequency (Fig 2B and Data Supplement, Figs S3A and S3C). Familial history of cancer was reported in 12/48 (25%) patients (Data Supplement, Table S3).

Eight SNVs in *APC* (n = 1), *PTEN* (n = 1), and *TP53* (n = 6) genes were detected in 6/26 (23.1%) patients with age <30 years (Fig 2B). All patients had a *TP53* mutation. Two patients had two co-occurring variants in HA-CSG genes, and 3/6 (50%) had at least one potentially germline variant belonging to the other classes (MA/SA). Two patients had breast cancer, two patients had CNS cancer, one patient had a GI stromal tumor, and one patient had colorectal cancer (Data Supplement, Fig S3C). Two of six (33.3%) patients reported familial history of tumor, whereas 2/6 (33.3%) did not. For two patients, the information was not available (Data Supplement, Table S3).

Finally, we categorized 110 mutations in 11 genes (51 Indels and 59 SNVs) in 103 patients in the SA-CSGs class (Fig 2C). ATM (34/110, 30.9%), NF1 (24/110, 21.8%), and BAP1 (22/110, 20%) were the most common mutated genes (Fig 2C).

Mutations belonging to the SA-CSG class were most frequent in biliary tract cancer (30/103, 29.1% of patients with SA-CSGs class variants; 30/271, 11.1% of the biliary tract cancer cohort). SA-CSG class variants were found in 27/342 (7.9%) patients with lung cancer and 11/107 (10.3%) patients with esophagogastric cancer (Fig 2C and Data Supplement, Figs

TABLE 2. Clinicogenomic Characteristics of Patients With or Without Potentially Germline Variants

Characteristic	All (N = 1,339)	No PGVs (n = 1,146)	PGVs (n = 193)	*P*
Age, years, median (range)	62 (18-89)	63 (18-89)	59 (18-82)	.0033
Patients with age <30 years		21	10	.3547
Sex, No. (%)				.536376
Male	715 (53.4)	616 (53.7)	99 (51.3)	
Female	624 (46.6)	530 (46.3)	94 (48.7)	
Tumor mutational burden, No. (%)				
Determined	1,225 (91.5)	1,045 (91.2)	180 (93.3)	.333081
Not determined	114 (8.5)	101 (8.8)	13 (6.7)	
Median (range)	4 Mut/Mb (0-216)	3 Mut/Mb (0-100)	4 Mut/Mb (0-216)	.0001
Family history for cancer, No. (%)				
Yes	262 (19.6)	223 (19.4)	39 (20.2)	.3764
No	389 (29.05)	341 (29.7)	48 (24.9)	
NA	688 (51.4)	582 (50.8)	106 (54.9)	

Abbreviations: NA, not applicable; PGV, potentially germline P/LP variant.

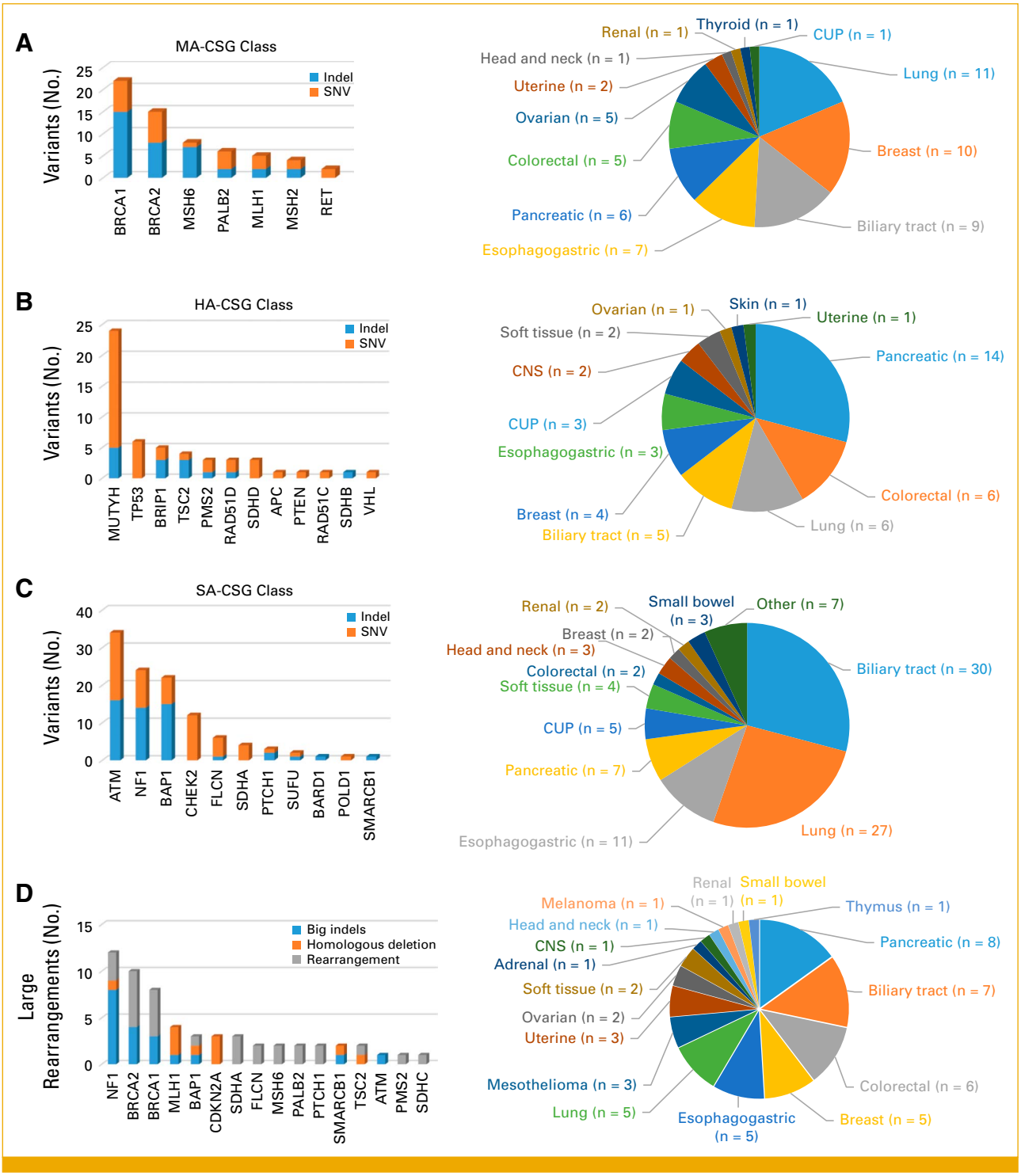

FIG 2. Categorization of potentially germline variants in CSG classes. (A) Potentially germline variants (n = 62) in genes classified in the MA-CSG class. Distribution of 59 patients with variants classified in the MA-CSG class across the tumor types. (B) Potentially germline variants (n = 53) in genes classified in the HA-CSG class. Distribution of 48 patients with variants classified in the HA-CSG class across the tumor types. (C) Potentially germline variants (n = 110) in genes classified in the SA-CSG class. Distribution of 103 patients with variants classified in the SA-CSG class across the tumor types. Other: anal cancer (n = 1 patient); adrenal cancer (n = 1 patient); liver cancer (n = 1 patient); CNS cancer (n = 1 patient); melanoma (n = 1 patient); ovarian cancer (n = 1 patient); and thyroid cancer (n = 1 patient). (D) LRs detected in the 16/40 CSGs in patients enrolled in the RATIONAL study. Distribution of patients with LRs across the tumor types. CSG, cancer susceptibility gene; HA, high actionable; LR, large rearrangement; MA, most actionable; SA, standard actionable.

S3A and S3D). Familial history of cancer was reported in 19/103 (18.4%) patients (Data Supplement, Table S3).

Analysis of LRs in CSGs

CGP allows for the detection of LRs, including deletions of the whole or partial gene, duplications, triplications, and inversions, in addition to SNVs and indels. In the whole cohort of patients, 58 LRs in 16 CSGs were detected, including 29 gene rearrangements (duplications, triplications, and inversions), 10 deletions of the whole gene deletions, and 19 partial gene deletions, in 53/1,339 (3.95%) patients. GAs in *NF1* were most frequently observed (12/58; 20.7%), followed by alterations in *BRCA2* (10/58, 17.2%) and *BRCA1* (8/58, 13.8%; Fig 2D).

The majority of LRs were detected in genes classified in the SA-CSG class (28/58; 48.3%), followed by genes in the MA-CSG class (26/58; 44.8%; Data Supplement, Fig S4). Among 53 patients with LRs, 33.9% of patients had familial history of tumor, 22.6% of patients did not, whereas for 43.4% of patients, the information was not available. Patients with pancreatic cancer had the higher number of potentially germline LRs (8/53, 15.1% of patients with LRs; 8/165, 4.8% of patients with pancreatic cancer), followed by patients with biliary tract cancer (7/53, 13.2% of patients with LRs; 8/271, 2.9% of patients with biliary tract cancer; Fig 2D). Eight patients (8/53; 15.1%) had concomitant potentially germline variants in genes belonging to other CSG classes. The majority of LRs were identified by tumor tissue testing (37/53; 69.8%).

Patients Referred for Genetic Counseling

The 225 potentially germline variants were identified in patients enrolled in 26 centers. Only 13/26 (50%) centers were able to provide data on germline testing and follow-up for 99/193 (51.3%) patients (Fig 3). Only 4/99 (4%) patients were referred to genetic counseling: two patients with biliary tract cancer in whom CGP detected variants in two genes of the MA-CSG class (*MLH1* and *BRCA1*), one patient with gastric cancer and familial history of tumor with a *NF1* variant and one patient with colorectal cancer carrying an *ATM* mutation.

Among the 95/99 (96%) patients not referred to oncogenetic consultation and follow-up, 30 carried variants in the MA-CSG class. Poor conditions or death within 3 months of report issuing (18/95; 18.9%), genetic counseling before the test (5/95; 5.3%), the lack of a geneticist in the center (5/95; 5.3%), and the lack of follow-up (5/95; 5.3%) are the reasons for which germline analysis and follow-up was not performed. However, reasons were not specified for 61/95 patients (64.2%; Fig 3).

DISCUSSION

Tumor sequencing using large panels is rapidly becoming an integral part of care of patients with metastatic cancers for identifying actionable mutations with therapeutic relevance. However, tumor-only sequencing also allows the identification of potentially germline variants associated with increased cancer risk.[8,9,13-16] Although these findings may not have implications for the treatment of the patient undergoing testing, the identification of potentially germline variants has extreme relevance for family members. Identifying a genetic risk of cancer allows the carrier to follow health surveillance programs and possibly undergo interventions for risk reduction.[17,18]

Obviously, there is a risk of the overinterpretation of tumor sequencing results. For this reason, ESMO has released recommendations to limit the impact of tumor-only findings, defining both the genes of interest and a VAF threshold associated with the probability that the identified variants may actually be germline.[11] When applying these rules, we found that about 14% of patients with cancer who received CGP had putative germline variants. Studies of tumor-only sequencing reported the presence of potentially germline variants in percentages of patients ranging from 3.6% to 40%.[11,13-15,19,20] Different factors, including the patients tested, the genes selected and the filters applied, and the approaches used for sequencing, may have a relevant impact on the rates of patients with potential germline variants. In this regard, ESMO guidelines recommends germline follow-up of *MUTYH* only for biallelic pathogenic variants. The inclusion of all *MUTYH* variants in our study may lead to overestimate the rate of patients with variants in CSGs. However, recent studies suggested that the presence of monoallelic *MUTYH* variants associated with somatic loss of heterozygosity might increase the susceptibility to some cancer types.[21] In addition, the American College of Medical Genetics and Genomics guidelines acknowledge that some *CHEK2* missense variants, including the I157T, should not be routinely reported on tumor or germline tests.[22] In our study, we found the *CHEK2* I157T missense variant in seven patients, one of whom also had a *BRCA2* variant. Germline follow-up is not recommended in carriers of this variant and, more generally, the management of *CHEK2* heterozygotes should be guided by personalized risk estimates.

Only two studies reported data obtained from the same CGP tests used in our study.[14,20] A study in 710 Japanese patients with advanced cancers reported a 40% rate of potential germline findings, whereas in a large cohort (n = 125,128) of patients with cancer, potential germline variants were detected in 9.7% of patients. However, in the first study, potentially germline variants in 46 recommended CSGs were described,[20] whereas in the latter manuscript, only 24 CSGs were considered using as cutoff VAFs > 10% for tissue CGP and >30% for liquid CGP. The high variability in the frequency of germline variants is likely due to the heterogeneous criteria adopted in these studies.

In agreement with previous findings,[14] the detection rate of GAs in CSGs was higher in patients tested on tissue compared with ctDNA. We do not expect that tissue and ctDNA testing

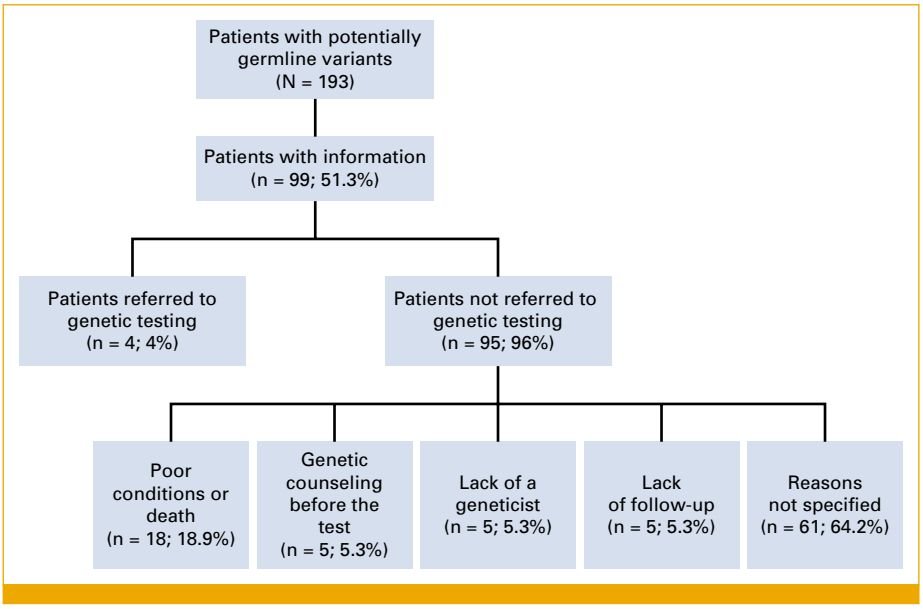

FIG 3. Diagram of referrals for germline testing of patients (n = 193) with potentially germline variants identified by tumor-only sequencing.

might show difference for the detection of germline variants. However, in cases with very low circulating cell-free DNA input, the sensitivity of the test could be affected. In addition, we noted that the majority of patients with breast (74%) and ovarian (86%) cancers were tested on tissue. Since these tumors have the highest frequency of mutations in CSGs, this imbalance may have contributed to the reported differences.

Alterations in CSGs, including MA-CSG group genes, have been identified both in tumors that are routinely subjected to genetic testing, such as ovarian and breast cancers, and in tumors that do not usually undergo genetic surveillance, such as cancer of unknown primary, cholangiocarcinoma, and lung cancer. Although our data are incomplete and may weaken our conclusions, many patients with CSG alterations did not have a family history of cancer, and therefore would never have undergone genetic testing. This observation highlights the potential of CGP, which could allow the identification of families with a genetic risk of developing cancer.

The off-target identification of CSG alterations has also been described in previous studies and raises an important question about their possible pathogenic role.[14,23] There is no evidence to support a role of off-target mutations in CSGs, such as *BRCA* variants identified in lung cancer or cholangiocarcinoma, in the development of these tumors. However, this possible correlation is worth investigating to clarify any pathogenic mechanisms not yet identified.[24]

Most previous studies on CSGs have only analyzed SNVs and indels. However, inactivation of CSGs can also be determined by gene rearrangements.[25-27] LRs can be difficult to detect and may be underreported as a cause for hereditary cancer risk. A previous study described that LRs account for approximately 7% of pathogenic variants identified by testing germinal DNA with a pan-cancer NGS panel.[28] Our study shows that LRs in CSGs can be detected by tumor tissue testing at a frequency of almost 4% in patients with cancer. Importantly, 44.8% of LRs occurred in genes of the MA-CSG class, thus confirming the relevance of detecting and reporting LRs.

The identification of individuals with a genetic risk of developing cancer could represent an important added value of the CGP.[29,30] However, we found that the majority of Italian oncologists are not trained to adequately manage this information. Only 4% of patients with potentially germline GAs were referred to genetic counseling, highlighting a significant lack of awareness of the relevance of this information among the Italian oncology community. The inclusion of *MUTYH* and low-risk *CHECK2* variants in our study might lead to overestimate this phenomenon. The ESMO recommendations indicate that genetic counseling is strongly recommended for all patients with alterations of the MA-CSG class, leaving the decision for the other classes to the oncologist on the basis of the organization of the national health system.[11] However, even considering only patients with MA-CSG alterations, 93.75% of patients did not receive genetic counseling. The RATIONAL study provides a snapshot of the Italian clinical practice, providing recommendations on the interpretation of the test result, but leaving management at the local level.[31] Our findings suggest that the results of CGP tests should always be discussed within molecular tumor boards or within multidisciplinary teams, where all the professional skills needed to interpret complex genomic data are available, including molecular

biologists and geneticists. Several evidences suggest that oncologists not only in Italy are trained in recognizing actionable mutations for therapeutic intervention and generally have a low ability to interpret complex genomic analyses.[32] In this respect, structured pathways for the management of germinal findings to support oncologists are not present in the majority of Italian oncology centers. Automated pipelines for predicting somatic versus germline origin and homozygous versus heterozygous or subclonal state of variants identified by tumor-only CGP have been recently developed that might help physicians by highlighting potentially germline variants.[33] A specific training of the oncologists to manage the complex implications of potentially genomic findings and a close collaboration with geneticists and molecular biologists are definitely required.

In conclusion, the data from the RATIONAL study confirm the utility of CGP also for the identification of alterations in CSGs and highlight the importance of reporting and paying attention to LRs in addition to SNVs and Indels. However, our findings also demonstrate a relative lack of knowledge of the implications of CGP tests among oncologists and underline the need for specific training in this area.

AFFILIATIONS

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