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Original Research

Current practice of genomic profiling of patients with advanced solid tumours in Italy: the Italian Register of Actionable Mutations (RATIONAL) study

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Abstract ***Background:*** The Italian Register of Actionable Mutations (RATIONAL) is a multicentric, observational study collecting next-generation sequencing (NGS)-based tumour profiling data of patients with advanced solid tumours.
Methods: The study enrols patients who had available an NGS-based tumour profiling (Pathway-A) or undergo comprehensive genomic profiling (CGP) with FoundationOne CDx assays within the trial (Pathway-B). The primary endpoint was the rate of actionable mutations identified.
Results: Sequencing data were available for 738 patients in Pathway-A (218) and -B (520). In Pathway-A, 154/218 (70.6%) tests were performed using NGS panels ≤52 genes, and genomic alterations (GAs) were found in 164/218 (75.2%) patients. In Pathway-B, CGP revealed GAs in 512/520 (98.5%) patients. Levels I/II/III actionable GAs according to the European Society of Medical Oncology Scale for Clinical Actionability of molecular Targets (ESCAT) were identified in 254/554 (45.8%) patients with non-small-cell lung cancer, cholangiocarcinoma, colorectal, gastric, pancreatic and breast cancer. The rate of patients with level I GAs was similar in Pathways A and B (69 versus 102). CGP in Pathway-B revealed a higher number of patients with level II/III GAs (99 versus 20) and potentially germline pathogenic/likely pathogenic variants (58 versus 15) as compared with standard testing in Pathway-A. In patients with cancer of unknown primary, CGP detected OncoKB levels 3B/4 GAs in 31/58 (53.4%) cases. Overall, 67/573 (11.7%) of patients received targeted therapy based on genomic testing.
Conclusion: The Italian Register of Actionable Mutations represents the first overview of genomic profiling in Italian current clinical practice and highlights the utility of CGP for identifying therapeutic targets in selected cancer patients.

1. Introduction

Precision oncology offers the opportunity for cancer patients to access targeted therapies, based on specific molecular and genomic features of their disease [1,2]. The number of agents targeting genomic biomarkers is constantly growing, due to the increased knowledge of cancer biology and the advancement in molecular diagnostics techniques [3,4]. Next-generation sequencing (NGS) technologies which allow the simultaneous analysis in several genes of a broad spectrum of genomic alterations (GA), including single nucleotide variants (SNVs), small insertions/deletions (indels), copy number variations and fusions, are increasingly employed for biomarker testing in clinical practice [5–7]. Comprehensive genomic profiling (CGP) with large NGS panels, usually covering hundreds of genes, may also provide information on complex biomarkers, including tumour mutational burden (TMB), microsatellite instability and homologous repair deficiency.

Although guidelines recommend using NGS for genomic profiling of at least some selected tumour types in clinical practice [6,8], we recently reported that the uptake of NGS is limited in several European countries including Italy [9]. The Italian Register of Actionable Mutations (RATIONAL) has been promoted by the Federation of Italian Cooperative Oncology Groups (FICOG) to collect NGS-based tumour profiling data. The primary aim of the study is to describe the frequency of actionable mutations within Italian patients undergoing genomic profiling with NGS. This study also offers the possibility to describe the use of NGS in the current clinical practice in Italy.

2. Patients and methods

2.1. Study design and participants

RATIONAL 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 44 participating institutions. All patients provided written informed consent. Although the study is focused predominantly on somatic alterations, patients are informed about all GAs, including those that could impact family risk, unless patients opt out.

2.2. Study workflow

The study enrolled 876 patients between October 2018 and August 2021 in two distinct pathways of enrolment. In Pathway-A, patients with any type of advanced solid tumour, who already received an NGS-based tumour profiling are registered. In Pathway-B, patients for whom no access to NGS is available and with specific clinical and pathological features, are offered access to the FoundationeOne CDx assays on either tumour tissue or blood samples. Enrollment in Pathway-B was limited to

patients with *EGFR* and *ALK* wild-type non-small-cell lung cancer (NSCLC), biliary tract cancer, cancer of unknown primary (CUP) and patients with any tumour type who progressed on targeted therapy. The detailed study design is provided in Supplementary Methods.

2.3. Data collection

Mutational (excluding variants of unknown significance) and clinical-pathologic (including age, sex, tumour type and treatments) data are collected in an electronic database. In Pathway-A, data of patients who underwent genomic profiling were retrospectively extracted from medical records, whereas for patients in Pathway-B, genomic data were prospectively collected. Clinical follow-up data were retrospectively collected at fixed time points (every 6 months).

2.4. Statistical analysis

The analysis of data in the study was mainly descriptive. Significance was determined using two-tailed Student *t*-test or Chi-square test. *P* values < 0.05 were considered statistically significant.

3. Results

3.1. Patients

Overall, 218 patients were enrolled in Pathway-A and 658 in Pathway-B. In Pathway-B, in which the enrollment was prospective and failed analyses were registered, tests failed for 113/658 cases (17.2%), with a failure rate per centre ranging between 3% and 42.8%. Inadequate quantity of the specimen (62/113 cases; 54.9%) or of DNA yield (30/113; 26.5%) or failure during testing (21/113; 18.6%), were the reasons for the failure of the analysis with the FoundationOne assays. Lung cancer (56 cases), CUP (21 cases) and biliary tract cancer (17 cases) were the most frequent tumour types for which the analysis was not successful. Small biopsies (fine needle aspiration) and cytology specimens from bronchial, pulmonary and hepatic sites were most frequently associated with test failure (82.3% of failed tests). Results were not available for additional 23 cases. Therefore, sequencing data were available for 740 patients enrolled in Pathway-A (n. 218) and B (n. 522) (Fig. 1). Two patients in Pathway-B did not meet the inclusion criteria (age < 18 years) and they were excluded from further analyses. The main characteristics of the 738 evaluable patients are summarised in Table 1 and Supplementary Fig. 1.

3.2. Genomic profiling of patients in Pathway-A

The clinical and pathological characteristics of patients enrolled in Pathway-A are summarised in Table 1. NSCLC

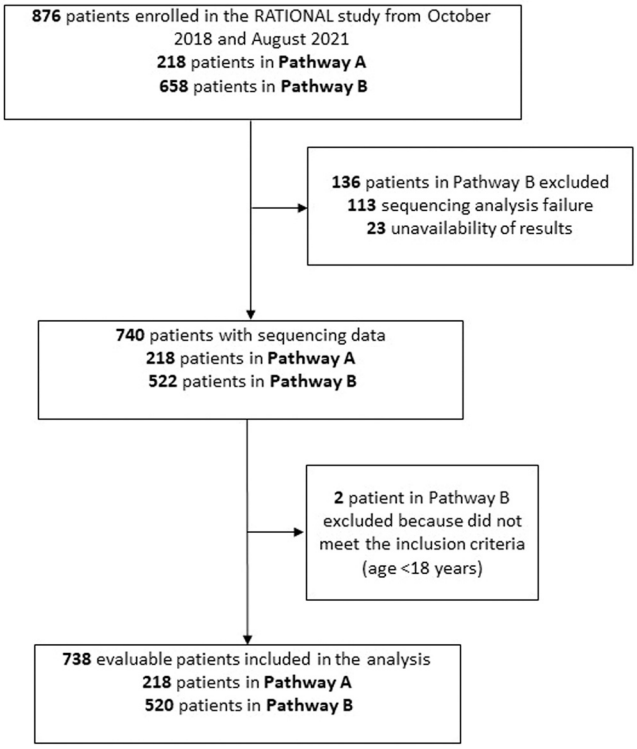

Fig. 1. Study workflow.

was the most frequent histology (150/218 cases; 68.8%), followed by colorectal cancer (CRC; 21/218; 9.6%) and cholangiocarcinoma (13/21; 6.0%). At the time of testing, 98/218 patients (45.0%) did not receive any treatment for metastatic disease, whereas 103/218 (47.3%) had at least one line of therapy, with a maximum of five (Table 1).

Genomic profiling was performed using different NGS panels (Supplementary Table 1). The majority of tests were performed on tissue (166/218; 76.2%) than in plasma samples (52/218; 23.8%). Overall, 154/218 (70.6%) tumour or liquid biopsy tests in Pathway-A were performed with NGS panels covering ≤52 genes.

Testing revealed in 75.2% (164/218) patients at least one GA, with 97 cases (44.5%) having at least two co-mutations. In particular, 523 GAs in 140 genes were found, including 227 SNVs, 94 indels, 108 gene amplifications, 16 homozygous deletions, 56 truncations and 22 rearrangements (Fig. 2A). Genes showing a frequency of GAs ≥3 are shown in Fig. 2B.

The mean number of GAs was significantly higher among the 60 cases tested with FoundationOne CGP panels (362 GAs, mean 6.1) as compared with the 158 samples tested with smaller panels (161 GAs, mean 1.5) ($p = 0.0001$) (Supplementary Table 2 and Supplementary Fig. 2).

Nineteen potentially germlines pathogenic/likely pathogenic (P/LP) variants, according to the European Society of Medical Oncology (ESMO) recommendations [10], in *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *PALB2*, *PMS2* and *MUTYH* were detected in 15/218 (6.9%) patients. Importantly, 15 variants were detected in 11/60

Table 1
Characteristics of patients included in the study.

Characteristics	All, n = 738	Pathway A, n = 218	Pathway B, n = 520
Age, years, median (range)	63 (18–87 years)	66 (18–85 years)	62 (18–87 years)
Sex			
Male	391 (53.0%)	109 (50.0%)	282 (54.2%)
Female	347 (47.0%)	109 (50.0%)	238 (45.8%)
Lines of previous therapy			
0	210 (28.5%)	98 (45.0%)	112 (21.5%)
1	195 (26.4%)	44 (20.2%)	151 (29.1%)
≥2	312 (42.3%)	59 (27.1%)	253 (48.6%)
NA	21 (2.9%)	17 (7.8%)	4 (0.8%)
Tumour type			
Lung cancer	324 (43.9%)	154 (70.6%)	170 (32.7%)
NSCLC	312	150	162
Adenocarcinoma	264	134	130
Non-adenocarcinoma	48	16	32
SCLC	2	2	–
Sarcomatoid carcinoma	6	1	5
Adenoid cystic carcinoma	3	1	2
Carcinoma neuroendocrine NOS	1	–	1
Biliary tract cancer	142 (19.2%)	15 (6.9%)	127 (24.4%)
Cholangiocarcinoma	114	13	101
Gallbladder cancer	17	–	17
Ampulla of Vater carcinoma	6	1	5
Biliary tract cancer NOS	5	1	4
Cancer of unknown primary (CUP)	59 (8.0%)	1 (0.4%)	58 (11.1%)
Digestive tract carcinoma	56 (7.6%)	22 (10.1%)	34 (6.5%)
Colorectal cancer	49	21	28
Intestinal cancer	7	1	6
Esophago-gastric tract carcinoma	34 (4.6%)	1 (0.5%)	33 (6.3%)
Gastric cancer	21	1	20
Gastroesophageal junction cancer	8	–	8
Oesophageal cancer	5	–	5
Pancreatic cancer	31 (4.2%)	5 (2.3%)	26 (5.0%)
Pancreatic cancer	30	5	25
Pancreatic neuroendocrine carcinoma	1	–	1
Breast cancer	28 (3.8%)	8 (3.7%)	20 (3.8%)
Gynaecologic cancer	17 (2.3%)	7 (3.2%)	10 (1.9%)
Ovarian cancer	13	5	8
Endometrial cancer	2	1	1
Uterine cancer	1	–	1
Vulvar cancer	1	1	
Soft tissue cancer	11 (1.5%)	1 (0.5%)	10 (1.9%)
Sarcoma	5	–	5
Paraganglioma	3	–	3
Synovial sarcoma	1	–	1
Gastrointestinal Stromal Tumor (GIST)	1	–	1
Leiomyosarcoma	1	1	–
Head and neck cancer	10 (1.4%)	–	10 (1.9%)
Thyroid cancer	3	–	3
Parathyroid carcinoma	3	–	3
Nasopharyngeal carcinoma	2	–	2
Tonsil carcinoma	1	–	1
Adenoid cystic carcinoma	1	–	1
Liver cancer	6 (0.8%)	2 (0.9%)	4 (0.8%)
Hepatocellular carcinoma	5	2	3
Liver neuroendocrine cancer	1	–	1
Skin cancer	5 (0.7%)	–	5 (1%)
Melanoma	3	–	3
Merkel cell carcinoma	1	–	1

(*continued on next page*)

Table 1 (*continued*)

Characteristics	All, n = 738	Pathway A, n = 218	Pathway B, n = 520
Porocarcinoma	1	–	1
Central nervous system cancer	4 (0.5%)	1 (0.5%)	3 (0.6%)
Glioblastoma	3	1	2
Meningioma	1	–	1
Renal cancer	4 (0.5%)	–	4 (0.8%)
Genito-urinary tract cancer	2 (0.3%)	1 (0.5%)	1 (0.2%)
Bladder Cancer	2	1	1
Thymus cancer	2 (0.3%)	–	2 (0.4%)
Adrenal cancer	1 (0.1%)	–	1 (0.2%)
Pleural mesothelioma	1 (0.1%)	–	1 (0.2%)
Prostate cancer	1 (0.1%)	–	1 (0.2%)

NA, not available; NOS, not otherwise specified; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer.

(18.3%) patients analysed with CGP tests and only 4 alterations (3 *BRCA1/2* and 1 *PALB2* alteration) in 4/158 (2.5%) patients sequenced with smaller panels.

3.3. Genomic profiling of patients in Pathway-B

In Pathway-B, 518 patients were analysed with the FoundationOne CDx assay and 2 with FoundationOne Liquid CDx, which became available only in the last few months of enrollment. For sequencing analysis, the median turnaround time (TAT) for tissue samples was 10 calendar days, with a TAT ≤14 d for 86.5% of the tests, from the receipt of the specimen to the report date. NSCLC (162/520 cases; 31.1%), cholangiocarcinoma (101/520; 19.4%), CUP (58/520; 11.1%) and CRC (28/520; 5.4%) were the more frequent histological types (Table 1). Patients with no previous therapy were 112/520 (21.5%), while 404/520 (77.7%) received up to a maximum of 11 treatments before testing.

The majority of patients (512/520; 98.5%) had at least one GA. Two or more GAs were detected in 475 (91.3%) patients. Overall, 2682 GAs (903 SNVs, 385 indels, 714 gene amplifications, 257 homozygous deletions, 332 truncations and 91 rearrangements) in 250 genes were identified, with a mean of 5.2 (range 1–24) GAs for the patient (Fig. 2C). A representation of genes carrying a number of GAs ≥13 is depicted in Fig. 2D.

Seventy-one potentially P/LP germline GAs were found in 58/520 (11.2%) patients in Pathway-B. Of these, 23 were in *BRCA1/2*, 19 in genes possibly associated with Lynch syndrome (8 *MLH1*, 4 *MSH2*, 5 *MSH6*, 2 *PMS2* variants) and 29 variants in other genes associated with different cancer syndromes (9 *MUTYH*, 7 *PALB2*, 4 *TSC2*, 3 *BRIP1*, *2 SDHB* and 1 *RAD51D*, *RET*, *SDHA* and *VHL* alterations). Only 4/23 patients (17.4%), for which we had information about genetic counselling, were referred to oncogenetic consultation after molecular testing.

Finally, TMB value was available for 459/520 patients, with a median value of 4 mut/Mb (mean 6.5 mut/Mb; range 0–111).

3.4. Actionability of GAs

We categorised 2398 GAs of 554 patients with NSCLC, cholangiocarcinoma, CRC, gastric, pancreatic and breast cancer in Pathways-A (198) and -B (356), according to the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) [6,11]. Overall, we identified 324 GAs belonging to ESCAT levels I, II and III in 254/554 (45.8%) patients.

In Pathway-A, we found 96 actionable GAs (74 level I, 4 level II, 18 level III) in 84/198 (42.4%) patients, with percentages ranging from 14.3% (3/21) in CRC to 50% (75/150) in NSCLC (Supplementary Table 3). Among 84 patients with actionable GAs, 29 (34.5%) patients had 1 GA and 55 (65.5%) had 2 or more GAs. In Pathway-B, 228 actionable GAs (110 level I, 27 level II, 91 level III) were identified in 47.8% of the cases (170/356), with the majority of patients (165/170, 97.0%) showing at least 2 GAs. The lower frequency of actionable GAs was observed in CRC (10/28; 35.7%) and the highest in pancreatic cancer (19/25; 76%), while cholangiocarcinoma was found to carry actionable GAs in 37.6% of the cases (Supplementary Table 3). The rate of patients with level I alterations was similar in Pathway-A and -B (69 versus 102; $p = 0.13$). However, CGP in Pathway-B identified a higher number of patients with level II/III GAs as compared with standard testing in Pathway-A (99 versus 20; $p < 0.00001$) (Table 2 and Supplementary Fig. 3). In addition, 91 patients had a TMB value ≥10 mut/Mb, among the 511 patients with available TMB data in both pathways.

3.5. Genomic profiling of patients with CUP

Fifty-eight patients with CUP (58/738; 7.9%) received a CGP test within the study, while one patient in Pathway-A was tested with a smaller panel. In the subgroup of patients receiving CGP, the median age was 60 years (range 22–81), 19 patients had adenocarcinoma histology and 39 non-adenocarcinoma (Supplementary Table 4). Nineteen patients with CUP did not receive

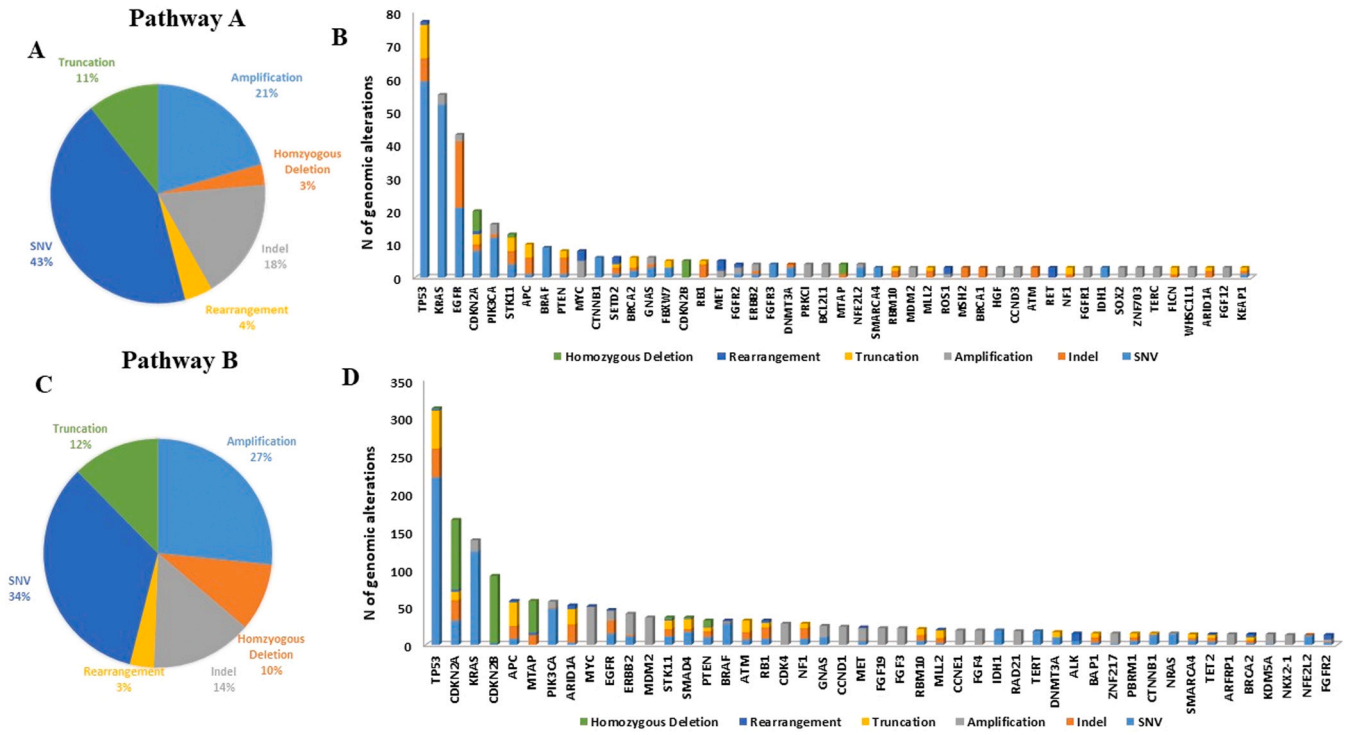

Fig. 2. Genetic variants identified in patients enrolled in Pathway A and B. A) Distribution of the different types of genomic alterations identified in patients enrolled in Pathway A. B) Genomic alterations identified in 218 patients enrolled in Pathway A. Genes with a number of genomic alterations ≥3 are shown. C) Distribution of the different types of genomic alterations identified with comprehensive genomic profiling in patients enrolled in Pathway B. D) Genomic alterations identified in 520 patients enrolled in Pathway B. Genes with a number of genomic alterations ≥13 are shown. indels, insertions and deletions; SNVs, single nucleotide variants.

therapy prior to molecular testing, whereas 39 patients had at least one treatment. Sequencing revealed the presence of at least one GAs in 56/58 (96.6%) patients. In detail, 237 GAs (84 SNVs, 27 indels, 62 gene amplifications, 39 truncations, 14 homozygous deletions and 11 rearrangements) in 104 genes were detected with an average of 4.2 GA/patient (Supplementary Fig. 4). TMB was evaluable for 53/58 patients, with values ranging from 0 to 72 mut/Mb (median 4.0; mean 6.60), and 8/53 patients (15%) showing a TMB ≥10 mut/Mb.

Overall, 47 potentially actionable GAs, classified in the levels 3B/4 of OncoKB [12], were detected in 31/58 (53.4%) patients, including 15/19 (78.9%) adenocarcinoma and 16/39 (41.0%) non-adenocarcinoma cases (Fig. 3 and Supplementary Fig. 5). In particular, 19 level-3B GAs were detected in 18/58 (31.0%) patients, whereas 28 level-4 GAs were found in 20/58 patients, including 7 patients with both level-3B and 4 GAs (Fig. 2).

3.6. Patients treated with matched targeted therapies

Follow-up data were available for 573/738 patients enrolled in the study. Overall, 67/573 (11.7%) patients received targeted therapy, based on the result of molecular analysis. In Pathway-A, 36/139 (25.9%) patients received matched targeted therapy, including 28/139 (20.1%) patients who were treated with a standard-of-care targeted agent and 8/139 patients (5.8%) who received targeted agents within clinical trials or other pathways (Table 3).

In Pathway-B, 31/434 (7.1%) patients were treated with targeted therapies based on CGP (Table 3). Among these, 10/434 patients (2.3%) received an approved targeted therapy, whereas 21/434 patients (4.8%) were treated with a targeted agent in clinical trials or with other modalities of accession.

Among 91 patients with TMB ≥10 mut/Mb, follow-up data were available for 35 patients. Twenty out of 35 patients (57%) received immunotherapy alone or in combination with chemotherapy. Among this group, 3/5 high-TMB patients with CUP (60%), for which post-

Table 2
Patients with or without ESCAT level I and levels II/III genomic alterations in Pathways A and B.

	Level I alterations (n. patients)		Level II/III alterations (n. patients)	
	With	Without	With	Without
Pathway A	69	129	20	178
Pathway B	102	254	99	257
	$p = 0.13$		$p < 0.00001$	

ESCAT, European Society of Medical Oncology Scale for Clinical Actionability of molecular Targets.

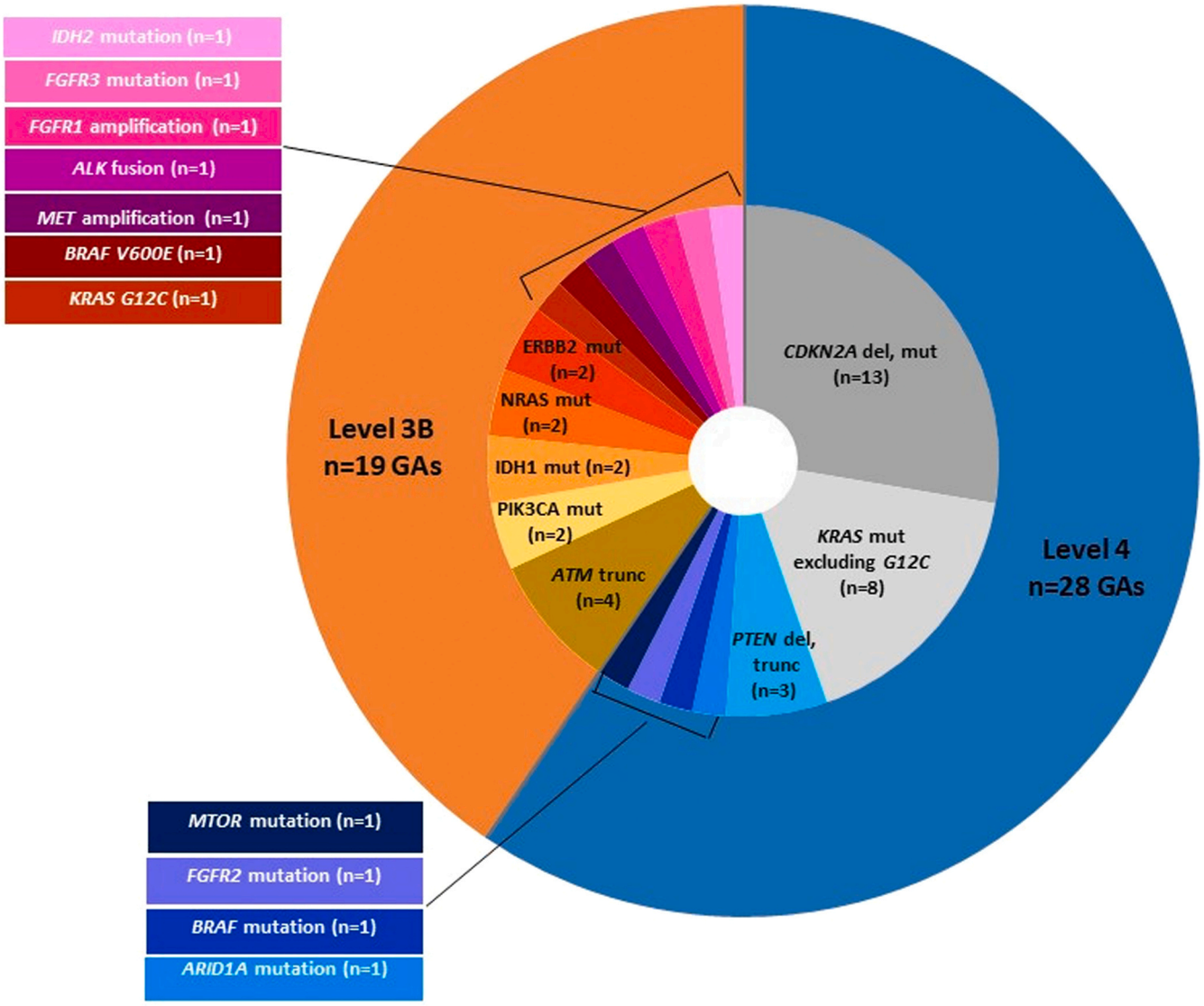

Fig. 3. Actionable genomic alterations in patients with CUP. Actionable genomic alterations in patients with CUP categorised in levels 3B and 4 according to the OncoKB database are shown. CUP, cancer of unknown primary.

analysis treatment data were available, received immunotherapy.

In order to assess the possible causes of the lack of targeted treatment for patients carrying actionable GAs, we also analysed the therapies received by patients with actionable GAs categorised according to ESCAT and listed in Supplementary Table 3. The patients with available follow-up data were 61 in Pathway-A and 146 in Pathway-B. In Pathway-A, 25 patients with actionable GAs did not receive therapy due to a lack of clinical trials or other pathways of access to drugs (n. 21; 84%) or poor clinical conditions or death at the time of the report (n. 4; 16%). Similarly, in Pathway B 16/115 patients (13.9%) died before the report was available, 32 (27.8%) died within 5 months suggesting poor clinical conditions and 67 (58.3%) did not receive targeted therapy because no drug was approved and reimbursed in Italy or clinical trials or other pathways of access to drugs were available, or patients already received suggested therapy in previous lines.

4. Discussion

Numerous prospective genomic screening programmes have been reported [13]. RATIONAL is a national programme of genomic profiling whose primary endpoint is the description of actionable GAs in Italian cancer patients. The rate of patients with ESCAT levels I/II/III GAs identified in the RATIONAL study was similar to previous studies, although we classified only GAs of the most frequent histologic subtypes present in our database [14,15].

The ESMO recommendations for NGS testing suggest using small panels covering only ESCAT level I

alterations in clinical practice [6]. Data collected in Pathway-A reveal that the use of large NGS panels for CGP is extremely limited in the current Italian clinical practice and more frequent in previously treated patients (Supplementary Table 2), while front-line testing is more frequently performed with relatively small panels covering a limited number of genes. In this respect, no significant difference was observed between Pathways-A and -B in the detection of level I GAs that are usually well covered by small NGS panels. However, CGP identified in Pathway-B a higher number of patients with level II/III GAs that offer potential for therapeutic intervention through clinical trials or other approaches. Importantly, CGP also detected co-mutations in the majority of patients carrying level I–III GAs. In this respect, evidence suggests that the presence of co-mutations might affect the outcome of patients treated with targeted therapies [16–20].

TAT of genomic profiling is a key issue to ensure that patients can receive targeted treatment. We did not collect data on TAT in Pathway-A. However, a recent survey published by our group found that the median TAT for NGS testing in reference centres in Italy is 28 d [9]. In contrast, the TAT in Pathway-B was only 10 calendar days, with a TAT ≤14 d for 86.5% of the tests, suggesting the feasibility of CGP in routine clinical practice.

The high number of failed analyses observed in Pathway B, mainly due to the inadequate quantity of tissue or DNA yield, evidences that the availability of tissue is one of the major limits for the use of CGP tests, which usually require higher levels of input DNA as compared with smaller panels [21]. These data highlight the need for the education of pathologists and laboratory personnel in charge of the selection of the tumour specimens to test. Our findings also highlight the need to improve all the steps involved in biomarker testing, starting from the collection of an adequate amount of tissue to ensure adequate genotyping of patients [22].

The fraction of enrolled patients who received a treatment matched to the GA in this study was only 11.7%, and a significant difference was observed between the two enrollment pathways (25.9% in A versus 7.1% in B). However, 20.1% patients received a standard of care regimen in Pathway-A versus 2.3% in Pathway-B. Such difference reflects the different characteristics of patients enrolled in Pathway-B, who were in most cases heavily pretreated, as compared with Pathway-A that enrolled mainly treatment naïve patients. In addition, Pathway-A was enriched of NSCLC patients for whom a high number of approved targeted therapies is available, as compared with Pathway-B (68.9% versus 31.1%). Finally, the enrollment in Pathway-A is retrospective and seems skewed towards the inclusion of patients with detected actionable GAs, while in Pathway-B enrollment and testing were prospective.

Overall, the fraction of patients who received a non-approved targeted therapy is close to 5%. These results are in line with previous studies in which although clinically relevant alterations were found in over 40% of patients, only 6–17% of cases received a matched genomic-driven therapy [14,15,23,24]. Several factors probably affect this result, including the limited number of clinical trials available, the difficulty in Italy of accessing drugs approved for different indications, and the poor performance status of patients who received a CGP test only after failure of multiple lines of therapy. An explanatory example of the difficulties in accessing the drug in Italy is represented by cholangiocarcinoma. Although level I–III alterations were found in 41 patients, only 5 received a targeted treatment mainly with different Fibroblast Growth Factor receptors (FGFR) within clinical trials. After approval by the European Medicine Agency, it takes up to 18 months before the approved drug is available and reimbursed by the Italian national health system. During this period, the drug can be obtained by a nominal request approved by the Ethics Committee or by Expanded Access Programmes which, however, require the approval of an experimental protocol in a limited number of centres. For off-label drugs, the only possibility of access is the request through a special fund of the Italian Medicines Agency for orphan drugs, which is extremely limited and only minimally covers the needs. These complex and limited options lead to a significant limitation of access to targeted agents in Italy.

An interesting finding of the RATIONAL study was that potentially germline P/LP GAs were found in 11.2% of patients in Pathway-B. The detection rate of potential germline GA was significantly lower in patients tested in Pathway-A with small panels, which do not cover genes associated with hereditary cancer syndromes. Previous studies have identified pathogenic germline variants in over 15% of cancer patients, suggesting that CGP might help to identify carriers of germline cancer-associated variants [25]. While this information might not have any impact on the management of the cancer patient, it has huge relevance for the families, providing the possibility of early diagnosis and preventive treatments. Tumour testing cannot determine whether a GA is somatic or germline. Guidelines recommend that when a potential germline P/LP GA is detected, the patient should be referred for genetic counselling [10]. This information was available only for 23 patients. Surprisingly, only 4/23 patients were referred to genetic counselling, underlying the need for the education of oncologists on the interpretation and use of data deriving from CGP.

The prognosis of patients with CUP is extremely poor due to the lack of adequate and specific treatments. In agreement with previous results [26–28], we identified OncoKb level 3B GAs in 31% of CUP, and a TMB ≥10 in eight cases. In a Phase II Randomized Study Comparing the Efficacy and Safety of Targeted Therapy or

Table 3
Matched targeted therapies and actionable GAs delivered to 67 patients enrolled in the study.

Actionable GA	ESCAT	Drug	N patients	Tumour type
Pathway-A				
ALK fusion	I	Alectinib	1	NSCLC
BRAFV600E	I	Dabrafenib + Trametinib	1	Cholangiocarcinoma
	I	Dabrafenib + Trametinib	1	NSCLC
EGFR Exon 20 insertion	II	Poziotinib	1	NSCLC
EGFR mutations	I	Afatinib, Osimertinib, Gefitinib	24	NSCLC
FGFR2 fusion	I	Pemigatinib	1	Cholangiocarcinoma
MET Exon14 skipping	I	Capmatinib	1	NSCLC
RET fusion	I	Selpercatinib, Pralsetinib	2	NSCLC
ROS1 fusions	I	Crizotinib	2	NSCLC
*RAS/BRAF*wt[a]	n.c.	Panitumumab + Folfiri	1	CRC
*RAS/BRAF*wt[a]	n.c.	Cetuximab + Irinotecan	1	CRC
Pathway-B				
ALK fusion	I	Lorlatinib	4	NSCLC
CDK12 rearrangement	IV	Rupacarib + Ipilimumab	1	Gastric cancer
EGFR Exon19del/ L858R	I	Osimertinib	3	NSCLC
ErbB2 amplification	I	Trastuzumab deruxtecan	5	Gastric cancer (n = 2), gastroesophageal junction cancer (n = 3)
ErbB2 amplification	II	Trastuzumab + Pertuzumab	1	Ampulla of Vater cancer
ErbB2 mutation	II	Poziotinib	1	NSCLC
ErbB2 mutation	II	Afatinib	1	NSCLC
FGF3-4-19 amplification	IV	Futibatinib	1	Cholangiocarcinoma
FGFR2 fusion	II	Derazantinib	1	Cholangiocarcinoma
FGFR2 rearrangement	II	Pemigatinib	1	Cholangiocarcinoma
KIT mutation	I	Imatinib	1	GIST
KRAS G12C	I	Sotorasib	2	NSCLC
LoH score	I	Niraparib	1	Ovarian cancer
MET Exon14 skipping	I	Tepotinib, Capmatinib	2	NSCLC
mTOR mutation,	n.c.	Everolimus	1	CUP
TSC2 mutation	n.c.	Everolimus	1	CUP
NF2 loss	IV	Everolimus + Octeotride	1	Meningioma
SDHB mutation	IV	Sunitinib	1	Paraganglioma
*RAS/BRAF*wt[a]	n.c.	Cetuximab	1	CRC
*RAS/BRAF*wt[a]	n.c.	Panitumumab + Irinotecan	1	CRC

CRC, colorectal cancer; CUP, cancer of unknown primary; GIST, Gastrointestinal Stromal Tumor; LoH, loss of heterozygosity; n.c., not classified; NSCLC, non-small-cell lung cancer.
[a] Resistance biomarkers.

Cancer Immunotherapy Versus Platinum-Based Chemotherapy in Patients With Cancer of Unknown Primary Site (CUPISCO) trial that employed the same FoundationeOne assay used in the RATIONAL study, 31.7% of patients with CUP could be matched to one of the treatment arms within the trial [29]. More recently, Möhrmann et al. found that up to 80% of CUP might receive genomics-based treatment recommendations by combining whole genome/exome, transcriptome and methylome sequencing [30]. Taken together, these data strongly support the use of CGP in the genomic profiling of CUP.

Although data suggest that high TMB might predict sensitivity to immune checkpoint inhibitors [31], this biomarker is not approved in Europe. Interestingly, 20/35 (57%) of patients with an available TMB test and values ≥10 mut/Mb received immunotherapy, including 3/5 patients with CUP. These findings suggest that this biomarker is somehow taken into consideration by oncologists for treatment decisions, if available.

In conclusion, the RATIONAL study represents the first overview of the use of cancer genomic profiling in current clinical practice in Italy. Our study provides a large dataset of GAs in patients with advanced cancers who might benefit from targeted agents. In addition, our data highlight the clinical utility of CGP for identifying therapeutic options in cancer patients, including cases with rare cancers, such as CUP.

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CRediT authorship contribution statement

NN, ADL, REA and CP contributed to conceptualisation, data collection, statistical analysis, original draft writing. AM, MM, FT, GC, CM, PM, GP, VG, GLF,

GF, VA, BD, RB, FF and EM contributed to patients' enrollment and data collection. All the authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: N. Normanno declares: speaker's fee and/or advisory boards from MSD, Bayer, Biocartis, Illumina, Incyte, Roche, BMS, Merck, Thermofisher, AstraZeneca, Eli Lilly; financial support to research projects (institutional grants) from Merck, Thermofisher, QIAGEN, Roche, AstraZeneca, Biocartis, Illumina; non-financial interests President of the International Quality Network for Pathology (IQN Path) and Past President of the Italian Cancer Society (SIC). G. Curigliano declares: speaker's fee/consulting fees/travel support from BMS, Roche, Pfizer, Novartis, Lilly, AstraZeneca, Daichii Sankyo, Merck, Seagen, Ellipsis, Gilead, Pfizer, Relay. V. Guarneri declares: speaker's fee/consulting fees/travel support from EliLilly, Amgen, GSK, Gilead, AstraZeneca, Eisai, Exact Sciences, Merck Serono, MSD, Novartis, Olema Oncology, Sanofi. V. Adamo declares: speaker's fee/consulting fees/travel support from Pfizer, MSD, Amgen, BMS, Novartis, AstraZeneca, Lilly. G. L. Frassineti declares: speaker's fee/consulting fees/travel support from Servier, Merck, Bayer, MSD. B. Daniele declares: speaker's fee/consulting fees/travel support from EISAI, Ipsen, AstraZeneca, ROCHE, Amgen, MSD, Sanofi. A. Morabito declares: speaker's fee/consulting fees/travel support from Roche, MSD, AstraZeneca, Novartis, Boehringer, Lilly, Takeda, BMS, Pfizer. G. Fasola declares: speaker's fee/consulting fees/travel support from Servier, Asytazeneca, Eubea, VyvaMed, MI&T, A&G, GSK, BMS, Merck, Ipsen. Roche, F. Tabbò declares: speaker's fee/consulting fees/travel support from Roche, AstraZeneca. Takeda, C. Masini declares consulting fees from BMS, Astellas, Janssen, MSD, Ipsen. C. Pinto declares personal fees from Amgen, Astellas, AstraZeneca, Bayer, Bristol Meyer Squibb, Celgene, Clovis Oncology, Eisai, Ipsen, Janssen, Incyte, Merck-Serono, Merck Sharp and Dohme, Novartis, Roche, Sandoz, Sanofi, and Servier, A. De Luca declares no conflict of interest. R. Esposito Abate declares no conflict of interest. M. Milella declares grants or contracts from Roche and Novartis, payment for consulting fees from Viatris, AstraZeneca, MSD, and Merck; payment for participation on a Data Safety Monitoring Board or Advisory Board from Novartis. P. Marchetti PM declares consultant/advisory role for BMS, Roche, Genentech, MSD, Novartis, Amgen, Merck Serono, Pierre Fabre, and Incyte. G. Pruneri declares honoraria from Novartis, Roche, Lilly and Exact Science. R. Berardi declares consultant/advisory board activities for AstraZeneca, Boehringer Ingelheim, Novartis, MSD, Otsuka, Eli-Lilly, Roche. F. Feroce declares no conflict of interest. E. Maiello declares no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.03.027.

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