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Feasibility of next-generation sequencing in clinical practice: results of a pilot study in the Department of Precision Medicine at the University of Campania 'Luigi Vanvitelli'

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ABSTRACT

Background The emerging role of next-generation sequencing (NGS) targeted panels is revolutionising our approach to cancer patients, providing information on gene alterations helpful for diagnosis and clinical decision, in a short time and with acceptable costs.

Materials and methods In this work, we evaluated the clinical application of FoundationOne CDx test, a hybrid capture-based NGS. This test identifies alterations in 324 genes, tumour mutational burden and genomic signatures as microsatellite instability. The decision to obtain the NGS assay for a particular patient was done according to investigator's choice.

Results Overall, 122 tumour specimens were analysed, of which 84 (68.85%) succeeded. The success rate was influenced by type of specimen formalin-fixed paraffin embedded (FFPE block vs FFPE slides), by origin of the sample (surgery vs biopsy) and by time of fixation (<5 years vs ≥5 years). The most frequent subgroups of effective reports derived from colorectal cancer (25 samples), non-small-cell lung cancer (16 samples), ovarian cancer (10 samples), biliary tract cancer (9 samples), breast cancer (7 samples), gastric cancer (7 samples). The most frequent alterations found in whole population referred to *TP53* (45.9%), *KRAS* (19.6%) and *APC* (13.9%). Furthermore, we performed an analysis of patients in whom this comprehensive genomic profiling (CGP) had a relevance for the patient's disease.

Conclusions On our opinion, CGP could be proposed in clinical practice in order to select patients that could most benefit from the analysis proposed, like patients with good performance status without any available treatments or with unexpected resistance to a therapy.

Key questions

What is already known about this subject?

► Comprehensive genomic profiling (CGP) is revolutionising the field of precision medicine in oncology. In particular, a great number of next-generation sequencing (NGS) panels able to identify genes alterations are now available allowing the detection of potential druggable alterations. However, considering the lack of large prospective clinical trials that certified its clinical utility, the risks of overdiagnosis and increase costs without survival benefits are real.

What does this study add?

► We have evaluated the feasibility of using an NGS panel (FoundationOne CDx) in routine clinical practice in our department of Precision Medicine. After an analysis of success rate in either overall population and in different subgroups, we have evaluated whether the genomic alterations were relevant for each single patient.

How might this impact on clinical practice?

► Our work shows that CGP could be proposed in clinical practice with a particular attention to patient's selection in order to maximise the clinical benefit.

INTRODUCTION

In the past years, the identification of gene alterations in solid tumours and the development of specific drugs against them, have formed the cornerstone of so-called 'precision medicine' in medical oncology.[1 2] Nowadays, over 100 targeted cancer drugs indications were recommended by Food and Drug Administration since the first approval of trastuzumab for treatment of human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer (BC).[3] Concurrently, the advance of diagnostic tools to detect these genetic alterations became

necessary. Indeed, a great number of comprehensive genomic profiling (CGP) tests were developed during last years with consequent reduction of the prices and their integration in clinical practice.[4] Among these, next-generation sequencing (NGS) plays a crucial role. This technique is able to sequence long sequences of DNA in a short time.[5] In fact, differently from other cheaper techniques, NGS covers a huge number of base pairs with a good sensitivity, less than digital pathological complete response (PCR) but more than Sanger sequencing. There are three main types of NGS sequencing: whole-genome sequencing (WGS), whole-exome sequencing (WES) and targeted sequencing (TS). In the first one, all coding and non-coding regions of DNA are sequenced, in the second one the exonic regions and the third only the targeted regions are sequenced. The lower costs and the higher depth of TS (until 10 000x and higher), makes it particularly suitable for the discovery of new druggable targets and a lot of commercial or 'in house' panels have been developed during last decade.[6] FoundationOne CDx (F1CDx) is a TS NGS-based diagnostic device for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations in 324 genes and identification of select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumour mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumour tissue specimens.[7] In this work, we evaluated the feasibility and clinical application of a CGP made with F1CDx in our oncology department. Analyses were performed on a total number of 122 FFPE tumour tissue specimens. We assessed total success rate and success rate in various subgroups according to type and age of sample tissue. Furthermore, we investigated all genetic alterations in the six most represented tumours of the study population: colorectal cancer (CRC), non-small-cell lung cancer (NSCLC), ovarian cancer (OC), biliary tract cancer (BTC), BC, gastric cancer (GC). Finally, we performed an analysis of patients in whom this CGP has had a relevant utility in clinical practice.

MATERIALS AND METHODS

Patients' characteristics

Patients provided informed consent for an institutional review board-approved protocol for collection of their archival tumour tissue and CGP using Foundation Medicine platform within the I-Cure research programme. Between 1 September 2018 and August 31 2019, 122 samples from 114 patients (≥18 years old) were sent to Foundation Medicine. Of these, only 10 samples derived from non-metastatic tumour. Patients were selected according to investigator's choice based on the following criteria: young patients (<45 years) or patients without any other approved therapy available or patients who did not respond to standard therapies according to their clinical pathological and molecular characteristics.

If the laboratory did not complete the analysis, another sample of the same patient was sent without further costs. There were two main reasons for failed analysis: insufficient tissue for analysis (TIFA) or lab fail (FMI lab fail) due to technical reason (eg, RNA degraded).

F1CDx assay

F1CDx is performed in a single site at Foundation Medicine. The test required ≥40 µm of FFPE tissue (5×5 mm^2). It could be both cytological or histological in 10 blank slides of 4 µm or in a paraffin block. In addition, adequate tissue (0.6 mm^3), tumour content (≥20%) and enough nucleated cells are required to proceed with the assay. The sample must yield a minimum of 55 ng of genomic DNA to ensure enough DNA for quality control (QC) and to proceed with library construction. In total, the assay detects alterations in a total of 324 genes. Using the Illumina HiSeq 4000 platform, hybrid capture-selected libraries are sequenced to high uniform depth (targeting >500X median coverage with >99% of exons at coverage >100X).[8] Additionally, genomic signatures including MSI and TMB are reported. To determine MSI status, 95 intronic homopolymer repeat loci (10–20 bp long in the human reference genome) with adequate coverage on F1CDx Assays are analysed for length variability and compiled into an overall MSI score via principal components analysis. Each sample is assigned a qualitative status of MSI-High (MSI-H) or MSI-Stable.[8] TMB is measured by counting all synonymous and non-synonymous variants present at 5% allele frequency or greater and filtering out potential germline variants according to published databases of known germline polymorphisms including Single Nucleotide Polymorphism database and Exome Aggregation Consortium. The resulting mutation number is then divided by the coding region corresponding to the number of total variants counted or 793 kb. The derived number is communicated as mutations per Mb unit (mut/Mb): low TMB for 1–5 mut/Mb, intermediate TMB for 6–19 mut/Mb, high TMB for ≥20 mut/Mb).[8] Approved results are annotated by automated software with CDx relevant information and are merged with patient demographic information.

RESULTS

Samples description

From 1 September 2018 to 31 August 2019, 122 tissue samples were collected in our cancer centre and were used for the analysis. Characteristics of the patients are shown in table 1. Caucasian population was included in the study and there were no differences between men and women. Median age was 59 years, mean was 58.2 years. Majority of patients had performance status 0 or 1 according to ECOG. The F1CDx was performed at baseline in 20% of patients and after first line of therapy in 50% of them. Tumour sample types are shown in table 2. Out of 122 samples, 8 were cytological samples (6.56%), while other 114 (93.44%) were histological ones. Among

Table 1 Characteristics of the patients' population. ECOG PS: Eastern Cooperative Oncology Group Performance Status

	Total N (%)	CRC	NSCLC	OC	BTC	BC	GC	Others
Age								
Median	59	54	62	53.5	67	62.5	60.5	56.5
Mean	58.2	53	59.6	55.6	68	59.5	58.4	54.3
Gender								
Male	61	14	24	0	9	0	5	9
Female	61	17	6	12	6	10	3	7
Race								
Caucasian	122	31	30	12	15	10	8	16
ECOG PS								
0	65 (53.3)	16	21	7	1	7	6	7
1	48 (39.3)	14	8	4	10	3	2	7
≥2	9 (7.4)	1	1	1	4	0	0	2
No of previous systemic anticancer therapies at the time of the test								
0	25 (20.5)	2	3	7	1	6	2	4
1	61 (50)	13	19	4	12	1	5	7
≥2	36 (29.5)	16	8	1	2	3	1	5

BC, breast cancer; BTC, biliary tract cancer; CRC, colorectal cancer; GC, gastric cancer; NSCLC, non-small-cell lung cancer; OC, ovarian cancer.

the cytological samples (all derived from NSCLC), seven were collected from primitive tumour, one was collected from a metastatic site. Instead, among the 114 histological samples, 101 were collected from primary tumour, while 13 were collected from a metastatic site (of which 1 was collected from a lymph node). Regarding the origin of the histological samples, 40 derived from a biopsy and 74 from a surgery. Moreover, 85 samples were FFPE tissue block and 29 were FFPE slides.

Samples subgroup analysis

Overall, 84 samples out of 122 completed the analysis (success rate: 68.85%). Among the 38 samples (31.15%) that failed the analysis, in 20 samples (52.63%) there was not enough TIFA, while in 18 samples (47.37%) the Foundation Medicine laboratory was not able to complete the analysis (FMI Lab Fail). For cytological samples only one out of eight reached the success rate (12.5%). Among the histological samples, 83 out of 114 (72.81%) completed the analysis whereas among the 31 failed (27.19%), 15 failed for TIFA and 16 for laboratory fail.

Similar fail rate was observed between samples collected from primitive tumour (26.73%) and those collected from a metastatic site (30.77%). On the contrary, considering samples derived from major surgery compared with biopsy-derived samples, a statistically significant difference in success rate was observed (79.73% vs 60% respectively, p=0.041). Furthermore, among the histological

Table 2 Anatomopathological diagnosis of the 122 samples

30 Non-small-cell lung cancer	26 colorectal cancer (23 adenocarcinoma, 2 carcinoid)
13 Biliary tract cancer	12 ovarian cancer (10 high-grade serous carcinoma, 1 germinal tumour, 1 yolk sac tumour)
10 Breast cancer (9 not otherwise specified +1 breast angiosarcoma)	7 gastric adenocarcinoma
2 oesophageal cancer (1 squamous and 1 adenocarcinoma)	2 hepatocarcinoma
2 pancreatic adenocarcinoma	2 soft tissue sarcoma (1 leiomyosarcoma and 1 liver sarcoma)
1 squamous cervical cancer	1 glioblastoma
1 liver hepatoid carcinoma	1 salivary gland tumour (parotid)
1 prostate adenocarcinoma	1 clear cell renal cell carcinoma
1 squamous cell vaginal cancer	

Five colorectal samples, two cholangiocarcinoma and one cervical cancer were resent because other samples of the same tumour were available after the first failure.

samples, we found also a significant difference between success rate of FFPE blocks compared with FFPE slides (78.82% vs 55.17% respectively, p=0.026). In particular, a higher lab fail rate has been found with FFPE slides underlining the importance of freshness of the sections' cut. Yates's X^2 test was used to evaluate differences between subgroups. Finally, we analysed whether the time of collection could influence the analysis. Samples were fixed between 2004 and 2019. Among 107 samples fixed within last 5 years, 74 samples (69.16%) completed the analysis, while 32 samples (30.84%) failed (18 for TIFA and 14 for FMI lab fail). Among 15 samples fixed more than 5 years, 10 samples (66.67%) completed the analysis while 5 (33.33%) failed (1 for TIFA and 4 for lab fail).

Turnaround time

Totally, 105 reports (86.06%) were received within 14 days from shipment, with a progressive decrease in the delivery time in the last months. Fifteen reports had some issues in the data inserted by the physician (e.a. date of birth of the patient, date of sampling) and the analysis was put on hold until those issues were clarified. Two report delayed for the repetition of the analysis.

Characteristics of patient's population

The most frequent subgroups of effective reports derived from CRC (25 samples), NSCLC (16 samples), OC (10 samples), BTC (9 samples), BC (7 samples), GC samples (7 samples). An overall summary of gene alterations is shown in figure 1A (genes altered in a single sample were not shown). We also divided them according to the subtype of alteration (amplifications, substitutions/indels, gene truncation, gene deletion, rearrangements) in figure 1B. The most frequent alterations found in whole population referred to *TP53* (45.9%), *KRAS* (19.6%) and *APC* (13.9%). Four patients had both TMB-high and MSI-H signatures, while other two patients had only TMB-high tumour signature.

Furthermore, genetic alterations were divided according to the pathway belonged (RAS, WNT/APC, Homologous Recombination Repair, RTK, PI3K/PTEN/AKT/mTOR, hormone receptor, MMR, apoptosis regulation, transcriptional regulation, cell-cycle regulation, chromatin remodelling, RNA maturation, angiogenesis pathway, JAK/STAT, TGFβ pathway, TP53, SRC, RB, others) (figure 2A–F, online supplementary table 1A–F). The most frequent altered pathway was chromatin remodelling pathway (ARID1A, MLL2, SMARCA4, BCORL1, etc). In CRC, NSCLC and biliary cancer genetic alterations were mostly related to RAS pathway (*RAS*, *RAF* genes). Genes involved in PI3K/AKT/mTOR pathway were often mutated in biliary and BC. Homologous recombination pathway was involved in almost all the cases of ovarian (figure 2C) and BC (figure 2E). Finally, concomitant alterations in the six most represented tumours are shown in online supplementary table 2A–F.

Clinically relevant cases

Lastly, we evaluated the clinical application of F1CDx test. Overall, among the 84 successful reports, 70 (83.3%) could be enrolled into clinical trial (including phase III trials in 37 samples) based on their genetic alteration: this percentage, however, is only a 'potential' enrolment rate. In order to demonstrate clinical utility of F1CDx, we selected the most relevant cases for which, the test was essential to highlight crucial alterations (table 3). Some of these patients were enrolled in clinical trial or undergone to an off-label drug. An overall summary of all the targetable alterations with corresponding clinical trial is shown in online supplementary table 3.

DISCUSSION

The idea of being able to treat all patients, each with a drug suitable for the specific alterations of his tumour, is certainly attractive for oncologists and especially for patients. In our work, we evaluated the feasibility of

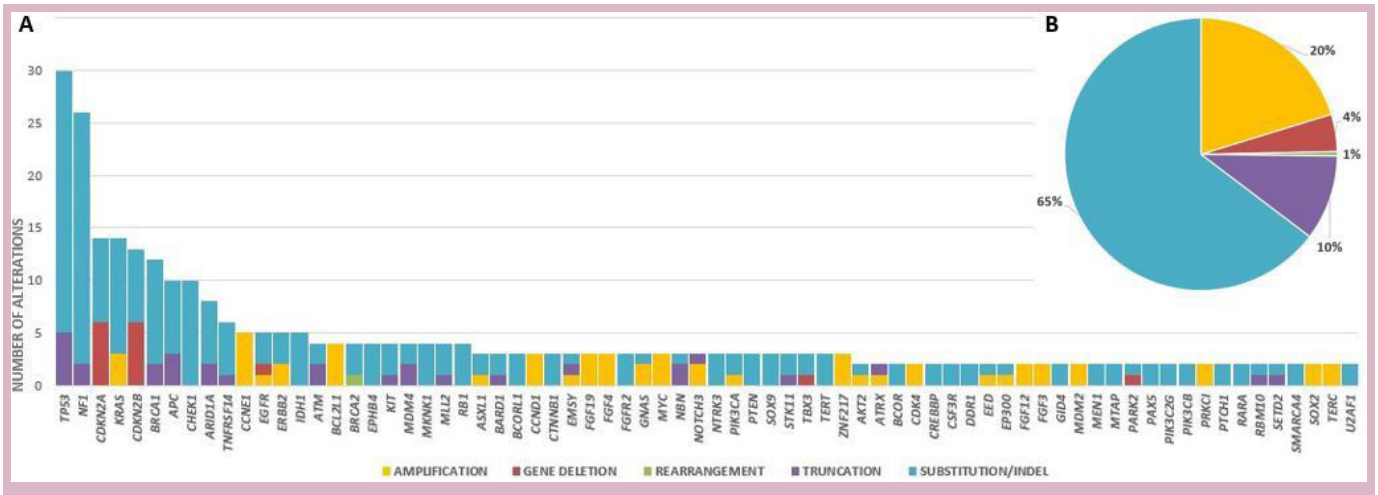

Figure 1 **(A)** Overall summary of gene alterations detected. Genes altered in a single sample were not shown. **(B)** Subtype of gene alterations found in the total population.

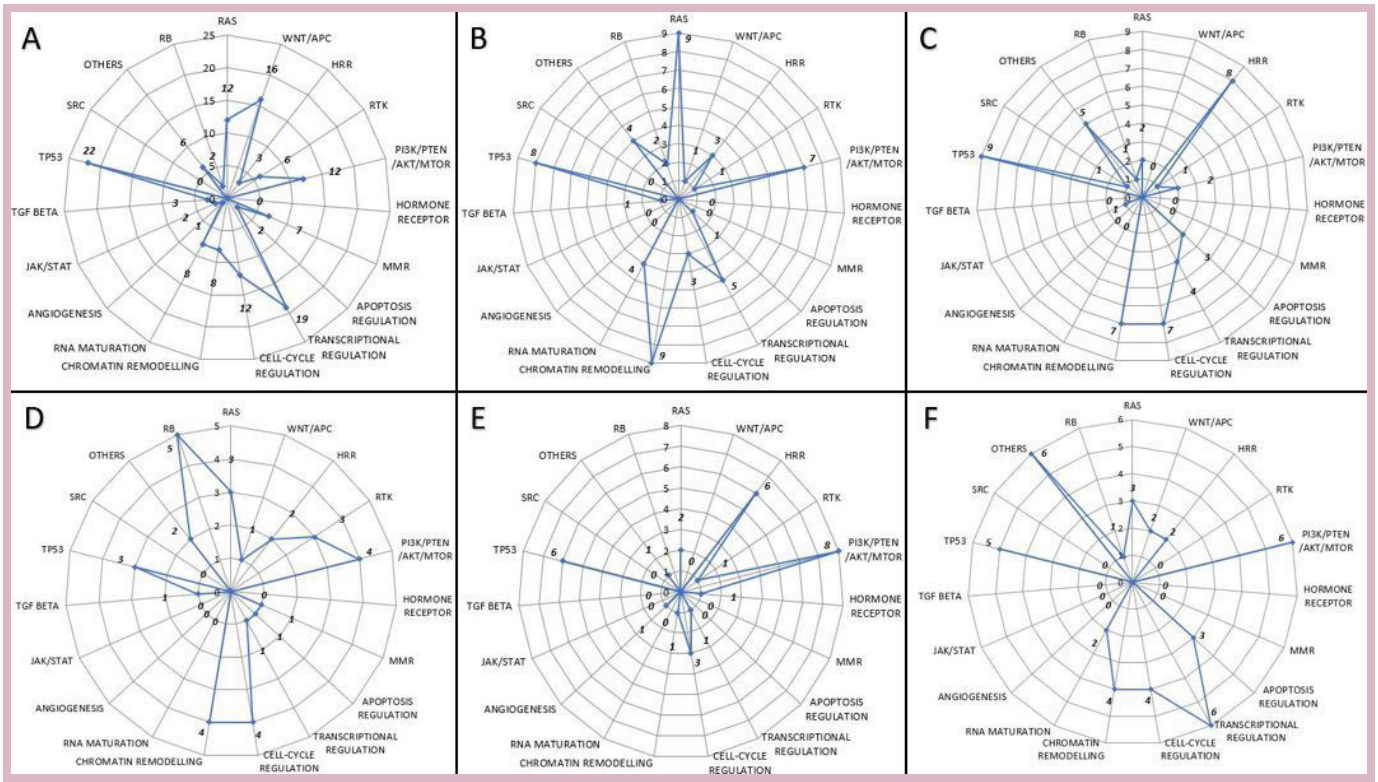

Figure 2 Radar charts according to pathways involved in the six most represented tumours. **(A)** colorectal cancer; **(B)** non-small-cell-lung cancer; **(C)** ovarian cancer; **(D)** biliary tract cancer; **(E)** breast cancer; **(F)** gastric cancer. TGF-β, transforming growth factor-β

clinical practice use of CGP performed with F1CDx in a heterogeneous population of patients from our institution.

First, we evaluated the overall success rate, which was lower (68.85%) than in other similar works,[9–12] due to several reasons. Foremost, in a significant percentage of cases, tissue qualification was not performed locally before the shipment: a local preassessment could potentially reduce the failure rate. Another reason could be the high heterogeneity of the sample's source (different time and type of fixation protocols from disparate peripheral centres). Subgroup analysis revealed that the 'ideal samples' to obtain the maximum yield should be histological samples derived from surgery, fixed recently (<5 years) in a FFPE block (success rate: 88%). Moreover, an advantage of sending FFPE blocks is that the FMI laboratories may be able to repeat the analysis if needed, while with slides samples, analysis couldn't be performed anymore.

Samples were sent at the discretion of our clinician, following the criteria described in the 'Material and methods' section. For this reason, type of tumour in the study population could not be representative of the general population (table 2). Furthermore, mean and median age are lower than those of worldwide patients with cancer.[13] We selected majority of patients (92.6%) with PS 0 or 1 according to ECOG to allow them to eventually undergo to experimental therapies. However, when the analysis was performed, at least one therapy was done in 79.5% of patients, including already Agenzia Italiana del Farmaco approved targeted therapies.

Regarding the clinical utility of this test in a real-world setting, we selected eight patients for which F1CDx revealed a treatment-changing alteration in the disease's history. Of these, only five patients already started an off-label therapy or participated in a clinical trial until now. However, the patients described in table 3 could be representative of the different situations that may arise after a CGP. In fact, among the five patients above mentioned, only one had a positive clinical outcome (pt 01, CR); the patients 02-03-04 started therapy few months before the data cut-off (one in an another hospital) and we do not know yet how targeted therapies work. Patient 05 started an off-label therapy with pembrolizumab but died few weeks later, underlining the necessity to perform CGP precociously during patient's history. The same concept applies to the patient 06 who, despite the presence of two alterations that could be targetable, was unable to participate in any clinical trial for his poor performance status. On the other hand, there are patients in which F1CDx test was done when there were still available therapies ongoing (pt 07) or non-metastatic patients in which CGP revealed essential information about prognosis and possible future therapies (pt 08). Indeed, 41 of the 84 successful reports derived from patients who are not progressed at the time of data cut-off, so they have not yet taken advantage of CGP but potentially could be recruited on clinical trials at the time of disease progression.

Table 3 Selected cases in which F1CDx offers therapeutic choices

Patient code	Age, sex	Diagnosis	Disease history	Alterations and signatures already known	Alterations and signatures detected by F1CDx	Comments
01	81 y, F	Stomach adenocarcinoma intestinal type with liver and bone metastases	▶ First line with FOLFOX ▶ Second line with Paclitaxel +Ramucirumab	▶ ERBB2 not overexpressed (IHC)	▶ **MSI-High** ▶ **TMB-High (58 muts/Mb)** ▶ AKT2 A139V ▶ BRCA1 K339fs*2 ▶ CDK12 T1463fs*50 ▶ PTCH1 N97fs*43 ▶ PTEN E157fs*23 ▶ RNF43 G659fs*41	After the second line the patient was in good clinical conditions but without any approved therapy. After the report of MSI status and TMB-high an off-label request for Nivolumab was done to our ethic committee based on the results of ATTRACTION-2 study.[34] Patient started therapy on October 2018 and after 3 months had a complete response which is maintained nowadays.[35]
02	29 y, F	Left colon adenocarcinoma with liver, lymph node and peritoneal metastases	▶ First line with FOLFOX +panitumumab ▶ Second line with FOLFIRI +ziv-aflibercept ▶ Third line with trifluridine/tipiracil	▶ KRAS wt ▶ NRAS wt ▶ BRAF wt ▶ ERBB2 not overexpressed (IHC)	▶ MS-Stable ▶ TMB-Intermediate (six muts/Mb) ▶ **AXL amplification** ▶ CCND2 amplification	AXL is a novel target in CRC.[36] In our department, a clinical trial with cabozantinib (a multikinase inhibitor of MET, RET, AXL, and VEGFR-2) in previously treated metastatic CRC patients is opened (EudraCT2019-000674-28). This patient was enrolled in this trial and treatment is ongoing.
03	66 y, M	Pancreatic adenocarcinoma with liver, bone and brain metastasis	▶ First line with FOLFIRINOX ▶ Second line with gemcitabine +abraxane	None	▶ **ETV6-NTRK3 fusion**	This patient without any other available therapy was found with NTRK3 fusion, allowing him to participate to STARTRK-2 study.[37] Treatment is ongoing.
04	76 y, F	Locally advanced intrahepatic cholangiocarcinoma	▶ First line with CDDP +Gemcitabine	None	▶ MS-Stable ▶ TMB-Low (five muts/Mb) ▶ **FGFR2-BICC1 fusion** ▶ CDK6 amplification	F1CDx revealed FGFR2-BICC1 fusion after the progression to first line therapy. She resulted eligible for the six trials according to the report. She went in another centre to participate to ARQ 087 trial (NCT03230318, ongoing).
05	54 y, M	Locally advanced left colon adenocarcinoma	▶ First line with FOLFOXIRI	▶ KRAS mut G12D ▶ BRAF wt	▶ **MSI-High** ▶ **TMB-High 25 muts/Mb**)	During the first line with FOLFOXIRI the patient had no clinical benefit and the PFS was only 6 months (Best Response: SD). Cardiovascular contraindications to anti-angiogenic therapies. An off-label request for Pembrolizumab was done according to KEYNOTE-164 results.[38] Unfortunately, he started therapy too late and after only one cycle, his performance status declined. He died few weeks later.
06	57 y, M	Intrahepatic cholangiocarcinoma with liver, lung and peritoneal metastases	▶ First line with CDDP +Gemcitabine ▶ Second line with FOLFIRI ▶ Third line with FOLFOX	None	▶ MS-Stable ▶ TMB-Low (0 muts/Mb) ▶ **ERBB2 amplification** ▶ **FGFR2-BICC1 fusion**	After the third line the patient was without any other available therapy. F1CDx revealed two druggable alterations with 15 trial proposed by the report. Unfortunately, the patient's clinical conditions worsened rapidly (PS 3 ECOG) and he has not been able to participate.
07	67 y, M	Intrahepatic cholangiocarcinoma with peritoneal metastases	▶ First line with CDDP +Gemcitabine	None	▶ MS-Stable ▶ TMB-Low (4 Muts/Mb) ▶ **IDH R132C**	F1CDx was performed during the first line therapy. A trial with an IDH inhibitor was available but the patient is still in maintenance with gemcitabine. Recently a positive trial was presented at ESMO 2019 with ivosidenib that improved PFS over placebo in IDH mutant cholangiocarcinoma.[39]

Continued

Table 3 Continued

Patient code	Age, sex	Diagnosis	Disease history	Alterations and signatures already known	Alterations and signatures detected by F1CDx	Comments
08	53 y, F	Infiltrating ductal carcinoma of the breast	▲ Quadrantectomy+level I/II axillary lymphadenectomy, stage pT2 N0 M0 ▲ Adjuvant therapy with EC90→ paclitaxel +RT	▲ Ki67 80% ▲ ER neg ▲ PgR neg ▲ ERBB2 not overexpressed (IHC)	▲ MS-Stable ▲ TMB-Intermediate (10 Muts/Mb) ▲ **BRCA1 W321*** ▲ CCND2 amplification ▲ FGF23 amplification ▲ FGF6 amplification ▲ KDM5A amplification ▲ RAD21 amplification ▲ TP53 P92fs*58	This patient is actually in follow-up but F1CDx revealed an important prognostic information and predictive in case of relapse.[40] Germline BRCA test is ongoing.

AXL, AXL receptor tyrosine kinase; BICC1, BicC Family RNA Binding Protein 1; BRAF, v-raf murine sarcoma viral oncogene homolog B1; CDDP, cisplatin; CDK6, cyclin-dependent kinase 6; CRC, colorectal cancer; ERBB2, v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2; ESMO, European Society for Medical Oncology; ETV6, Ets-leukemia virus; F, female; F1CDx, FoundationOne CDx; FGFR2, fibroblast growth factor receptor 2; IDH, isocitrate dehydrogenase; IHC, immunohistochemistry; KRAS, Kirsten rat sarcoma viral oncogene homolog; M, male; MET, tyrosine-protein kinase Met; MSI, microsatellite instability; NRAS, neuroblastoma ras viral oncogene homolog; NTRK3, neurotrophic tyrosine kinase receptor type 3; PFS, progression free survival; PTEN, phosphatase and tensin homolog; TMB, tumour mutational burden; y, year.

Moreover, most reports offered the possibility to participate in a clinical trial and more than half of them are phase III trials, which implies good evidence of clinical activity for the proposed drugs. We have also to consider that all the samples, even those sent at baseline, had already been subjected to routine molecular analysis (eg, RAS/RAF status in CRC, Epidermal growth factor receptor (EGFR) mutation in NSCLC, ERBB2 overexpression in GC) and almost all the breast and ovarian samples had a well-known BRCA mutation. For this reason, all these data were not included in the therapy-guiding information.

Besides, a major comprehension of the patient's tumour biology through this test, could be useful not only to identify an innovative therapy, but also to reveal mechanisms of sensitivity and resistance to previous treatments. In fact, we found a great number of useful information from several genomic reports. For example, in a patient with pCR after a first line with FOLFOX (leucovorin calcium, fluorouracil, and oxaliplatin)+panitumumab (stage IV rectum adenocarcinoma), F1CDx revealed an EGFR gene amplification that could explain the optimal response.[14] High TMB in two patients with NSCLC clarify the excellent RR and PFS.[15] In addition, in another work, F1CDx help us to explain various pattern of response to PARP inhibitor in four patients with BRCA-mutated high-grade serous OC: in particular, we noted two long responders (PFS=27 and 36 months) probably due to IDH mutation and PI3K with SOX2 amplification, respectively, and one with a very short PFS, possibly due to an NF1 mutation.[16]

Several studies assessed the clinical utility of CGP right with Foundation Platform. They found similar genes and pathway involved to our work with higher percentage of patients treated with genotype directed therapy (12%–35%).[9–12] Considering the time of follow-up of our study (in most of patients F1CDx was performed in the last 6 months of the study), and the fact that many patients did not progress to their ongoing therapies, probably we will reach this percentage during next years. Indeed, a good number of prospective[17–23] and retrospective[24–27] trials evaluated CGP using different techniques (NGS, WES, WGS) but in the context of large academic centres. Largest prospective study defined the potential and the limitations of extensive genomic panel (SHIVA,[17] NCI-MATCH,[18] NCI-MPACT,[19] ASCO-TAPUR,[20] I-PREDICT,[21] WINTHER,[22] PROFILER.[23] In these trials, similar to ours, major barriers to allow extensive CGP in all cancer patients were: high presence of alterations with limited clinical or only preclinical evidence; rapid disease progression after the analysis; spatial and temporal tumour heterogeneity that could affect outcomes. The emerging of liquid biopsy could overcome this question, also allowing to monitor the progress of specific alterations, as already assessed by various works.[28–32]

This study has several limitations: first of all, it was a retrospective evaluation and patients enrolled were chosen by physician-dependent criteria on the basis of what described in materials and methods (but this is what happen also in clinical practice); this latter question

influenced also the percentage of targetable alterations because patients with well-known driver mutations were not enrolled (eg, BRAF V600 mutation in melanoma, ERBB2 amplification in BC). Furthermore, a molecular tumour board was not set up to select the best therapy for the patients, even if it could help in most complex case to prioritise treatment options[33]; besides, no outcome analysis were done because of sample size and the short observation time.

In conclusion, CGP with F1CDx is feasible in clinical practice choosing accurately 'when' and 'which' are the samples to test to maximise the benefit. In fact, on our opinion, considering the lack of large prospective clinical trials that certified the clinical utility of CGP, this could be proposed also in clinical practice but with attention to timing and patient's selection. In particular, it should be performed in patients with still a good performance status (no more than 1) who have no more available approved treatments or patients with unexpected response or resistance to a therapy whose tumour could be driven by a rare and specific alteration. However, further studies are needed to avoid overdiagnosis and increase of the costs without real benefits in terms of improving survival or quality of life of our patients.

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Correction notice Surname of the of the third author has been corrected from 'Pietro Paolo Vitello' to 'Pietro Paolo Vitiello'.

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Contributors Conceptualisation: VDF, LP, PPV, TT and FCi; Data curation: LP, VDF, VC, FCa, RDL, AV and VF; Formal analysis: VDF and LP; Funding acquisition: FCi and TT; Investigation: LP, VDF, VC, FCa, RDL, AV and VF; Methodology, VDF, LP and TT; Resources: LP, VDF, VC, FCa, RDL, AV, VF and PPV; Supervision: TT, EM, FCa, FM, MO, FDV, MF, SN, GM and CMDC; Validation: TT, EM, FCa, FM, MO, FDV, MF, SN, GM, CMDC, RF and LA; Visualisation: VC, FCa, RDL, AV, VF, DC, PV, NZ, EFG, MT, CMDC, GM and SN; Writing—original draft: VDF and LP; Writing—review and editing: VDF, LP, TT and FCi.

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