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), oварian 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.

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. 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). 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. Among these, next-generation sequencing (NGS) plays a crucial role. This technique is able to sequence long sequences of DNA in a short time. 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 10000x 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. 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. 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.

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 mm²). 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 mm³), 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). Additionally, genomic signatures including MSI and TMB are reported. To determine MSI status, 95 intronic homopolymer repeat loci (10–20bp 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. 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 793kb. 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.

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

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.
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    | 2    | 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.

Table 2  Anatomopathological diagnosis of the 122 samples

| Diagnosis                                      | 122   |
|------------------------------------------------|-------|
| Non-small-cell lung cancer                     | 30    |
| Biliary tract cancer                           | 13    |
| Breast cancer (9 not otherwise specified +1 breast angiosarcoma) | 10    |
| Oesophageal cancer (1 squamous and 1 adenocarcinoma) | 2     |
| Pancreatic adenocarcinoma                      | 2     |
| Squamous cervical cancer                       | 1     |
| Liver hepatoid carcinoma                       | 1     |
| Prostate adenocarcinoma                        | 1     |
| Squamous cell vaginal cancer                   | 1     |

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 X² 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.g. 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, BCO2L1, 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
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, 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. 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.
| 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 | ERBB2 not overexpressed (IHC) | MSI-High | 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. Patient started therapy on October 2018 and after 3 months had a complete response which is maintained nowadays. |
| 02           | 29 y, F | Left colon adenocarcinoma with liver, lymph node and peritoneal metastases | First line with FOLFOX + panitumumab | KRAS wt | MS-Stable | AXL is a novel target in CRC. 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 | None | ETV6-NTRK3 fusion | This patient without any other available therapy was found with NTRK3 fusion, allowing him to participate to STARTRK-2 study. Treatment is ongoing. |
| 04           | 76 y, F | Locally advanced intrahepatic cholangiocarcinoma | First line with CDDP + Gemcitabine | KRAS mut G12D | MS-Stable | 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 wt | MSI-High | 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. 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 | None | MS-Stable | 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 | 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. |
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 (e.g., 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. High TMB in two patients with NSCLC clarify the excellent RR and PFS. In addition, in another work, F1CDx helped 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.

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%). 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 and retrospective 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, NCI-MATCH, NCI-MPACT, ASCO-TAPUR, I-PREDICT, WINHER, PROFILER). 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.

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; besides, no outcome analysis were done because of sample size and the short observation time.

In conclusion, CGP with FICDx 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 and VF; 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, NM, 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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REFERENCES
1 Berger MF, Mardis ER. The emerging clinical relevance of genomics in cancer medicine. Nat Rev Clin Oncol 2018;15:353–65.
2 Hyman DM, Taylor BS, Baselga J. Implementing Genome-Driven oncology. Cell 2017;168:584–99.
3 Sun J, Wei Q, Zhou Y, et al. A systematic analysis of FDA-approved anticancer drugs. BMC Syst Biol 2017;11:87.
4 Borad MJ, RoRusso PM. Twenty-First century precision medicine in oncology: genomic profiling in patients with cancer. Mayo Clin Proc 2017;92:1583–91.
5 Yip S, Christofides A, Baenerji S, et al. A Canadian guideline on the use of next-generation sequencing in oncology. Curr Oncol 2019;26:e241–54.
6 Bewicke-Copley F, Arjun Kumar E, Palladino G, et al. Applications and analysis of targeted genomic sequencing in cancer studies. Comput Struct Biotechnol J 2019;17:1348–59.
7 Frampton GM, Fletcher JS, Tan-Chiu E, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013;31:1023–31.
8 FoundationOne®CDx Full Specification Information. Available: https://www.accessdata.fda.gov/ cdrh_docs/pdf17/P170009C.pdf
9 Hirshfield KM, Toikunov D, Zhong H, et al. Clinical Actionability of comprehensive genomic profiling for management of rare or refractory cancers. Oncologist 2016;21:1315–25.
10 Hilai T, Nakazawa M, Hodskins J, et al. Comprehensive genomic profiling in routine clinical practice leads to a low rate of benefit from genotype-directed therapy. BMC Cancer 2017;17:602.
11 Johnson DB, Dahlman KH, Knol J, et al. Enabling a genetically informed approach to cancer medicine: a retrospective evaluation of the impact of comprehensive tumor profiling using a targeted next-generation sequencing panel. Oncologist 2014;19:816–22.
12 Wheler JJ, Janku F, Naing A, et al. Cancer therapy directed by comprehensive genomic profiling: a single center study. Cancer Res 2016;76:3690–701.
13 National Cancer Institute. Age and cancer risk. Available: https://www.cancer.gov/about-cancer/causes-prevention/risk/age
14 Khan SA, Zeng Z, Shia J, et al. Egrf gene amplification and KRAS mutation predict response to combination targeted therapy in metastatic colorectal cancer. Pathol Oncol Res 2017;23:673–7.
15 Greillier L, Tomasini P, Barlesi F, et al. The clinical utility of tumor mutational burden in non-small cell lung cancer. Transl Lung Cancer Res 2018;7:639–46.
16 Francese E, Centonze S, Diana A, et al. Genomic profile and BRCA-1 promoter methylation status in BRCA mutated ovarian cancer: new insights in predictive biomarkers of olaparib response. Front Oncol 2019;9:1289.
17 Le Tourneau C, Delord J-P, Gonzalves A, et al. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. Lancet Oncol 2015;16:1324–34.
18 ECOG-ACRIN. NCI-MATCH / EAY131 interim analysis, 2019. Available: https://ecog-acrin.org/nci-match-eay131/interim-analysis [Accessed 20 Nov 2019].
19 Chen AP, Williams C, Ramnarain S, et al. Feasibility of molecular profiling based assignment of cancer treatment (MPACT): a randomized NCI precision medicine study. JCO 2016;34:2539.
20 Mangat PK, Halabi S, Bruinooge SS, et al. Rationale and design of the targeted agent and profiling utilization registry (TAPUR) study. JCO Precis Oncol 2018;2:e2018-1.
21 Sicklick JK, Kato S, Okamura R, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. Nat Med 2019;25:744–50.
22 Rodland J, Soria J-C, Berger P, et al. Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. Nat Med 2019;25:751–8.
23 Trédan O, Wang Q, Pissaloux D, et al. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLERT trial. Ann Oncol 2019;30:757–65.
24 Nesline MK, DePietro P, Dy GK, et al. Oncologist uptake of comprehensive genomic profile guided targeted therapy. Oncotarget 2019;10:4616–29.
25 Laes J-F, Attimos P, Barthelemy P, et al. The clinical impact of using complex molecular profiling strategies in routine oncology practice. Oncotarget 2018;9:20282–93.
26 Dalton WB, Forde PM, Kang H, et al. Personalized medicine in the oncology clinic: implementation and outcomes of the Johns Hopkins molecular tumor board. JCO Precision Oncology 2017:1–19.
27 Naito Y, Takahashi H, Shitara K, et al. Feasibility study of cancer genome alterations identified by next generation sequencing: ABC study. Jpn J Clin Oncol 2018;48:559–64.
28 Campos CDM, Jackson JM, Witek MA, et al. Molecular profiling of liquid biopsy samples for precision medicine. Cancer J 2018;24:93–103.
29 Lissa D, Robles Al. Comprehensive genomic analysis of circulating tumor DNA for patients with advanced non-small cell lung cancer. Ann Transl Med 2019;7:80.
30 Le D, Kavan P, Kim T, et al. Safety and antitumor activity of pembrolizumab in patients with advanced microsatellite instability–high (MSI-H) colorectal cancer: KEYNOTE-164. Annals of Oncology 2018;29:v107.
31 Drilon A, Sankhala KK, Liu SY, et al. Abstract CT060: STARTRK-2: a global phase 2, open-label, basket study of Entrectinib in patients with locally advanced or metastatic solid tumors harboring Trk, ROS1, or ALK gene fusions. Cancer Res 2017;77:CT060.
33 van de Haar J, Hoes L, Voest E. Advancing molecular tumour boards: highly needed to maximise the impact of precision medicine. ESMO Open 2019;4:e000516.
34 Kang Y-K, Boku N, Satoh T, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017;390:2461–71.
35 Tirino G, Petrillo A, Pompella L, et al. Durable complete radiological response to nivolumab in two heavily pretreated Western elderly patients with metastatic gastric cancer: a case report. Front Oncol 2020;10:130.
36 Artiglio B, Martini G, Cardone C, et al. Axl is an oncotarget in human colorectal cancer. Oncotarget 2015;6:23281–96.
37 Drilon A, Sankhala KK, Liu SY, et al. Abstract CT060: STARTRK-2: a global phase 2, open-label, basket study of Entrectinib in patients with locally advanced or metastatic solid tumors harboring Trk, ROS1, or ALK gene fusions. Cancer Res 2017;77:CT060.
38 Le D, Kavan P, Kim T, et al. Safety and antitumor activity of pembrolizumab in patients with advanced microsatellite instability–high (MSI-H) colorectal cancer: KEYNOTE-164. Annals of Oncology 2018;29:v107.
39 Drilon A, Sankhala KK, Liu SY, et al. Abstract CT060: STARTRK-2: a global phase 2, open-label, basket study of Entrectinib in patients with locally advanced or metastatic solid tumors harboring Trk, ROS1, or ALK gene fusions. Cancer Res 2017;77:CT060.