Implementation and use of whole exome sequencing for metastatic solid cancer

Manon Réda, Corentin Richard, Aurelie Bertaut, Julie Niogret, Thomas Collot, Jean David Fumet, Julie Blanc, Caroline Truntzer, Isabelle Desmoulins, Sylvain Ladoire, Audrey Hennequin, Laure Favier, Leila Bengrine, Julie Vincent, Alice Hervieu, Jean-Florian Guion Dusserre, Pascal Foucher, Christophe Borg, Juliette Albuissier, Laurent Arnould, Sophie Nambot, Laurence Faivre, Romain Boidot, Francois Ghiringhelli

Department of Medical Oncology, Georges François Leclerc Cancer Center - UNICANCER, 1 rue Professeur Marion, Dijon 21000, France
Platformation of Transfer in Cancer Biology, Georges François Leclerc Cancer Center - UNICANCER, Dijon, France
Université Bourgogne Franche-Comté, Dijon, France
Genomic and Immunotherapy Medical Institute, Dijon, France
INSERM U1231, Dijon, France
Methodology, Data-Management, and Biostatistics Unit, Georges François Leclerc Cancer Center - UNICANCER, Dijon, France
Private hospital Sainte Marie, Chalon sur Saône, France
Department of Thoracic Oncology, Dijon University Hospital, Dijon, France
Department of Medical Oncology, Besançon University Hospital, Besançon, France
Department of Tumour Biology and Pathology, Georges François Leclerc Cancer Center - UNICANCER, Dijon, France
Centre de Référence Maladies Rares "Anomalies du Développement et syndromes malformatifs", FHU-TRANSLAD, Dijon University Hospital, Dijon, France

Abstract

Background: Genomically-guided clinical trials are performed across different tumor types sharing genetic mutations, but trial organization remains complex. Here we address the feasibility and utility of routine somatic and constitutional exome analysis in metastatic cancer patients.

Methods: Exoma trial (NCT02840604) is a multicenter, prospective clinical trial. Eligible patients presented a metastatic cancer progressing after at least one line of systemic therapy. Constitutional genetics testing required geneticist consultation. Somatic and germline exome analysis was restricted to 317 genes. Variants were classified and molecular tumor board made therapeutic recommendations based on ESMO guidelines. Primary endpoint was the feasibility of the approach evaluated by the proportion of patient that received a therapeutic proposal.

Findings: Between May 2016 and October 2018, 506 patients were included. Median time required for tumor sample reception was 8 days. Median time from sample reception to results was 52 days. Somatic analysis was performed for 456 patients (90.1%). Both somatic and constitutional analyses were successfully performed for 386 patients (76.3%). In total, 342 patients (75%) received a therapeutic proposal. Genetic susceptibility to cancer was found in 35 (9%) patients. Only, 79 patients (23.1%) were treated with NGS matched therapy mainly PI3K/AKT/mTOR inhibitors 22 (27.8%), PARP inhibitors 19 (24.1%), antiangiogenics 17 (21.5%), MEK inhibitors 7 (8.9%) and immunotherapy 5 (6.3%). Matched treatment was finally stopped because of disease progression (>1,3 for 23.5% of patients).

Interpretation: Study shows that exome analysis is feasible in cancer routine care. This strategy improves detection of genetic predispositions and enhances access to target therapies. However, no differences were observed between PFS ratios of patients treated with matched therapy versus standard therapy.

Funding: This work was funding by the centre Georges Francois Leclerc

© 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license. (http://creativecommons.org/licenses/by-nc-nd/4.0/)
Research in context

Evidence before this study

Precision medicine is a new era in the field of cancer therapy. In many cancer types, driver mutations could be targeted by small molecules, leading to a high response rate and better survival. Such mutation could be found in many cancer type thus leading to the hypothesis that large molecular screening may help physician to find better treatment. Many clinical trials test this hypothesis in tertiary cancer center. These trials use large somatic NGS panel or whole exome. Advantage of Exome techniques is that this test will not require technical implementation if new genes have and can be used to find genetic predisposition to cancer. However, such strategy is complex because it requires a good organization between oncogeneticists and oncologists to improve patient information and care. The complexity of clinical NGS testing has prevented many hospitals and laboratories from routine usage such large genomic testing.

We here address the feasibility and utility of routine somatic and constitutional exome analysis in a prospective cohort of metastatic cancer patients that received at least one line of chemotherapy.

Added value of this study

This Study shows that exome analysis is feasible in cancer routine care. This strategy improves detection of genetic predispositions and enhances access to target therapies. However, such strategy did not improve patient outcome.

Implications of all the available evidence

The disappointing results of this study underline our superficial understanding of the mechanisms in cancer evolution and cancer heterogeneity. It also highlights the fact that even with the existing targeted agents, if our comprehension is probably superficial. Probably multomics strategies and the incorporation of novel technologies like RNA-sequencing, whole genome sequencing and circulating cell-free DNA detection should be emphasized for future studies in order to estimate the possibility of novel targets and potential agents for these targets. Alternatively, exploration of particular "extraordinary response" or surprising failure of target therapies may help us to better understand cancer biology.

1. Introduction

High-throughput next-generation sequencing gives a new insight in the molecular landscape of cancer. Molecular profiling underlines that a same tumor type can contain variable molecular subgroups with different molecular properties. Importantly, particular mutation and related active molecular pathways lead to the identification of druggable targets. Over recent years, based on this concept, oncology has served as a paragon for the application of clinical genomics to treatment of disease [1]. In many cancer types, driver mutations could be targeted by small molecules, leading to a high response rate and better survival. In certain cancer types like colorectal cancer, lung cancer and melanoma, molecular profiling has become standard practice to search for targetable mutations [2–4]. This work is currently translated in the concept of precision medicine where genomically-guided clinical trials have begun to evaluate the efficacy of molecularly-targeted therapies across different tumor types with shared genetic mutations [5].

Currently, clinical trials include large somatic NGS panel or using constitutional and somatic analysis of large panel genes or whole exome [6]. One advantage of Exome techniques is that this test will not require technical implementation if new genes have to be analyzed. The use of constitutional analysis is helpful to find genetic predisposition to cancer in addition to finding targeted therapies. However, such strategy is complex because it requires a good organization between oncogeneticists and oncologists to improve patient information and care. In addition, the analysis of somatic and constitutional mutations supports the discovery of unknown genetic variants present in tumor DNA. The targetable relevance of such mutations is not addressed at the present time. The complexity of clinical NGS testing has prevented many hospitals and laboratories from routine usage of large NGS analysis. Currently, molecular NGS profiling trials are only performed in expert centers because of complexity and time required for bioinformatics analysis.

We here address the feasibility and utility of routine somatic and constitutional exome analysis in a prospective cohort of metastatic cancer patients that received at least one line of chemotherapy.

2. Patients and methods

2.1. Study design and procedure

The Exoma trial (NCT02840604) is a multicentric, prospective clinical trial. The Trial was approved by the ethical comitee called (Comité protection des personnes Est). The trial accrued patients between May 2016 and October 2018. Participating principal investigators were located at Dijon Cancer Center (centre Georges Francois Leclerc), Dijon University Hospital, Besancon University Hospital and Chalon sur Saone Private Hospital (Clinique Sainte Marie). Genomic analyses were performed at the Georges-Francois Leclerc Cancer Center, in the Genomic and Immunotherapeutic Medical Institute, in Dijon. This study aimed to show that exome analysis is feasible in patient routine care and improves access to target therapies and detection of genetic cancer predisposition. Patients were eligible if they presented a locally advanced non-operable or metastatic cancer that had progressed during at least one line of systemic therapy. We only included patients with non-curable diseases. All patients provided a signed informed consent for the trial and genomic analysis. After informed consent, patients had a consultation with a geneticist to explain the consequences of a constitutional genetic testing. Only after this consultation patient could accept or refuse the blood sample for constitutional exome analysis. This trial protocol was approved by an institutional review committee and done in accordance with the Declaration of Helsinki. Study was reported according to CONSORT Checklist.

2.2. Sample selection

After signed informed consent, physicians selected an archival tumor sample of less than one year (primary or metastasis) for genomic analysis. At the discretion of the physician, a new tumor biopsy could be proposed to the patient. Tumor cellularity was assessed by a senior pathologist on a hematoxylin and eosin slide from the same biopsy core used for nucleic acid extraction and molecular analysis.

2.2.1. DNA isolation

DNA was isolated from archival tumor tissue using the Maxwell 16 FFPE Plus LEV DNA Purification kit (Promega, Madison, WI, USA). DNA from whole blood (germline DNA) was isolated using the Maxwell 16 Blood DNA Purification Kit (Promega) following the manufacturer’s instructions. Quantity of extracted genomic DNA was assessed by a fluorimetric method with a Qubit device.
2.3. Whole exome capture and sequencing

Two hundred ng of genomic DNA were used for library preparation, using the Agilent SureSelectXT reagent kit (Agilent Technologies, Santa Clara, USA). The totality of enriched library was used in the hybridization and captured with the SureSelect All Exon v5 or v6 (Agilent Technologies) baits. Following hybridization, the captured libraries were purified according to the manufacturer’s recommendations and amplified by polymerase chain reaction (12 cycles). Normalized libraries were pooled and DNA was sequenced on an Illumina NextSeq500 device using 2 × 111-bp paired-end reads and multiplexed. Tumor and germline DNA sequencing generated mean target coverages of 78X and 90X respectively, and a mean of more than 90% of the target sequence was covered with a read depth of at least 10X for somatic DNA.

2.4. Exome analysis pipeline

Raw DNA sequencing data were aligned to the hg19 genome build using the Burrows-Wheeler Aligner (BWA) version 0.7.15. Duplicates were marked with Picard version 2.5.0. Base quality scores recalibration and variant calling were performed using GATK tools version 3.6.

For SNV (Single Nucleotide Variation), annotation was performed using the VariantStudio Illumina software. Filters of candidate variants included: coverage depth of 10X or greater and a variant nucleotide allelic fraction in tumor DNA greater than 5%.

2.5. Determination of tumor mutational burden per Mb

Whole exome sequencing data were used to generate tumor mutation burden per Mb for each patient. Tumor mutational burden (TMB) corresponds to the total number of missense and indel somatic-specific mutations divided by the number of megabases (Mb) of the target sequences of the SureSelect All Exon v5 or v6 baits (≈ 50.6Mb). Tumor mutations were identified from paired exomes by subtracting SNV observed in germline exome from SNV observed in the corresponding somatic exome.

2.6. Analysis of somatic mutations

We limited our analysis to 317 genes (Table 1). This list is adapted from Foundation One [7]. We used the knowledge database of somatic mutations Cosmic v.64 released on 2013, March 26th, to classify each selected variant as ‘pathogenic’, ‘probable pathogenicity’ ‘unknown pathogenicity’, or ‘benign’ variants. For each detected and annotated variant we retained for interpretation only variants annotated as pathogenic or likely pathogenic. Unknown variants were retained when present in somatic analysis only and located in a critical domain of the protein. Each therapeutic proposal was then classified using a homemade classification approved by our molecular tumor board: Grade A: positive phase II or III, Grade B: no data in this disease but positive data in other disease or case reports, Grade C: in vitro experiments. After publication of ESCAT recommendation we replaced our grade by ESCAT because of their strong similarity [8].

2.7. Analysis of constitutional mutations

On the basis of the same list of 317 genes [7], we performed the analysis in both whole blood cells DNA and tumor DNA to determine whether gene variant was present constitutionally or only in tumor sample. We limited constitutional analysis on 26 genes upon recommendation of our geneticists coming from American College of Medical Genetics gene list (https://www-ncbi-nlm-nih-gov/clinvar/docs/acmg/) (Table 1). Filters of candidate variants included: coverage depth of 10X or greater and a variant nucleotide allelic fraction in tumor DNA greater than 5%.

When the analysis indicated possible cancer susceptibility, the results were given on the clinical reports and explained to the patient by a geneticist in order to offer adapted follow-up.

2.8. Statistical hypotheses and analysis

The Exoma trial aimed to show that exome analysis was feasible in patient routine care and improved access to target therapies and detection of genetic cancer predisposition. The primary objective was that more than 30% of included patient could receive a therapeutic proposal. In order to estimate this proportion with a precision of the 95% confidence interval of 4%, 506 patients will have to be included in the study.

In addition, to assess the clinical impact of NGS adjusted therapies, we examined clinical results, as in the Von Hoff model [9], the PFS2/PFS1 ratio for the patients treated after NGS analysis results. This ratio corresponds to the comparison of the progression-free survival on matched therapy (PFS2) with the progression-free survival for the most recent therapy, on which the patient had just experienced progression (PFS1). Progression-free survival on matched treatment (PFS2) was defined as the time from start of treatment to progression, as defined by RECIST 1.1, clinical progression, or death from any cause. Progression-free survival on prior therapy (PFS1) was defined as the time from start of the last prior treatment to progression as defined by RECIST 1.1 or clinical progression [10]. Each patient is his own control.

The matching score for each patients was calculated as the number of characterized DNA alterations affected by the drug (or drugs) proposed divided by the total number of characterized alterations.

The point estimates and the associated 95% CI were provided. Standard statistical tests including the chi-squared test and Fisher’s exact
test for categorical data and the t-test for continuous data were applied. PFS were analyzed by the Kaplan–Meier estimate. The log-rank test and Cox proportional hazards model (Wald test) were applied to test the effect of covariates on PFS. The assumption of the Cox regression was validated.

Statistical analyses were performed in SAS 9.4 and R 3.2.2.

3. Results

3.1. Population characteristics

Between May 2016 and October 2018, 506 patients were included in the EXOMA clinical trial. From this cohort, we could obtain tumor tissue and isolated DNA in 456 cases (90.1%). Flow-chart is represented in Fig. 1a.

The analysis could not be performed in 50 cases (9.9%), due to insufficient tumor content or DNA and we have excluded samples that did not meet post-sequencing quality control criteria. Altogether, we successfully sequenced somatic DNA for 456 patients (90.1%). For constitutive analysis, 452 patients (89.3% of all patients) met an oncogeneticist to be informed and give consent for the constitutional analysis; 54 patients (10.5%) did not (patient’s refusal). Among the 452 patients who had an oncogeneticist consultation, 16 patients (3.1%) refused the analysis and data was missing for 3 patients. In total, both somatic and constitutional analyses were available for 386 patients (76.3%).

We included a mean of 16.7 patients per month. The median time for reception of tumor sample was 8 days [0–379]. The median turn-around time from sample reception to results was 52 days [3-339]. For patients with available tumor sample, 385 samples (84.4%) came from archival sample. A new biopsy was performed for 71 patients (15.6%).

The main tumor type was breast cancer (21.5%), followed by colorectal (14.8%) and pancreatic cancer (14.2%) which reflected the classical recruitment of metastatic patients in including centers (Fig. 1b).

Table 2 summarizes the clinical characteristics of the included patients.

3.2. Landscape of constitutional and somatic mutations

For somatic analysis we limited our analysis to 317 genes (Table 1). The three most frequently tumor altered genes in the EXOMA cohort were TP53 (38.6%), KRAS (18%) and PIK3CA (13.8%) (Fig. 2a).

Among the 5 main cancers, TP53 mutations were the most prevalent: 52.9% of patients in colorectal cancer, 49.2% of patients in pancreatic cancer, 48.6% of patients with ovarian cancer, 35.9% of patients with breast cancer, and 33.3% of patients with NSCLC. TP53 mutations coding consequences were mainly missense variants (68%) and frameshift variants (16%).

KRAS mutations were the second most prevalent: 50.8% of patients with pancreatic cancer, 44.3% of patients with colorectal cancer and 23.3% of patients of NSCLC. KRAS mutations coding consequences were mainly missense variants (94%).

The third most prevalent mutations were PIK3CA mutations, present for 24.3% of patients with breast cancer and 13.5% for patients with ovarian cancer (Fig. 2b). PIK3CA mutations coding consequences were mainly missense variants (94%).

We could determine the tumor mutational burden (TMB) in 313 patients for which both somatic and constitutional exome analysis were available. TMB is the number of coding and non-coding mutations divided by the length of the sequencing design. The median TMB was 5.1 mutations per Mb (range 0.6–54). The tumor type with higher TMB was NSCLC, a median of 6 mutations per Mb. The tumor type with the lower TMB was the ovarian cancer with a median of 4.4 mutation per Mb p = 0.15 (Mann-Whitney test) (Fig. 2c). We observed a strong correlation between mutations in DNA repair genes (either somatic or constitutional) and TMB (Fig. 2d).

For 386 patients (76.3%) we could perform constitutional exome analysis. We limited our analysis on 26 genes known to be related to increase risk of cancer (Table 1). We observed 361 variants, 197 neutral or benign, 129 variants of unknown significance and 35 deleterious variants. Nine patients required a new consultation by a geneticist for cancer predisposition that were not discovered before inclusion in this clinical trial. Fig. 2e shows the impact of constitutional alterations in actionable genes.

3.3. Clinical actionability and utility

All WES analyses were discussed at the molecular tumor board. A therapeutic proposal was done if there was an open clinical trial testing a drug which targets the mutation, or if there was an approved drug available for the relevant disease or for another disease known to target the mutated gene or the related activated pathway. The decision was based on discussion made at the tumor board with a basic proposal established according to Target database (https://software.broadinstitute.org/cancer/cga/target), then decision was classified using international recommendations provided by ESMO Scale of Clinical Actionability for molecular Targets (ESCAT) [8]. In addition,
each mutation could have a particular biological impact which was categorized in four categories (i.e. pathogenic, likely pathogenic, unknown significance or benign) [11]. In most trials, only class I and II (pathogenic, likely pathogenic) were selected for therapeutic proposal, while class III (mutation of unknown significance) were excluded. In this study, class III variants were also retained for therapeutic proposal. As shown in Table 3, we selected for recommendation grade I to III ESCAT level of evidence and for some cases the choice, most of the time with standard of care for 66 patients (57.9%), death for 40 patients (35.1%) and palliative care for 114 patients (9.6%). In this cohort, 79 patients (23.1%) were treated at progression with a matched therapy; 114 patients (43.3%) were further untreated, due to palliative care.

Table 2
List of genes used for somatic and constitutional analyses. Genes displayed in red are those used for constitutional analysis.

| ABL1 | ABL2 | AKT1 | AKT2 | AKT3 | ALK | AMER1 | ANAPC2 | APC | AR |
|------|------|------|------|------|-----|-------|--------|-----|----|
| ARAF | ARFRP1 | ARID1A | ARID2 | ASXL1 | ATM | ATR | ATRX | AURKB | AURKB |
| AXIN1 | AXIN2 | AXL | BAP1 | BARD1 | BCL2 | BCL6 | BCR | BCR1L1 | BLM |
| BRAF | BRCAL | BRCA1 | BRIP1 | BRIP1 | CD79A | CD79B | CD25A | CD34 | CD73 |
| CCND1 | CCND2 | CCND3 | CCNE1 | CDK6 | CDK7 | CDKN1B | CDKN2A | CDKN2C | CDKN3 |
| CDK12 | CDK4 | CDK5 | CDK6 | CDK7 | CDK8 | CDKN1B | CDKN2A | CDKN2C | CDKN3 |
| CHEK1 | CHEK2 | CIC | CKIT | CREBBP | CRKL | CRLF2 | CSFR1 | CTCF | CTNNB1 |
| CPTNB1 | CUL1 | CUL2 | CUL3 | DAXX | DDR2 | DIERI | DNMT3A | DOT1L | DPDY |
| EZ2F1 | EORF | EORF | EORF | EORF | EORF | EORF | EORF | EORF | EORF |
| ESR2 | EZH2 | FAA46C | FANCA | FANC | FANCD2 | FANCE | FANC | FANC | FANC |
| FXB7 | FGF17 | FGF18 | FGF19 | FGF23 | FGF3 | FGF4 | FGF6 | FGFR1 | FGFR2 |
| FGFR3 | FGFR4 | PLCN | FLT1 | FLT3 | FLT4 | FOXL2 | GATA1 | GATA2 | GATA3 |
| GID4 | GI1 | GLZ | GLZ | GNA11 | GNA13 | GNAQ | GNAS | GREM1 | GREN2A |
| GRP | GSK3A | GSK3B | HGF | HRAS | HNSAP9A | IKB | IKB | IKB | IKB |
| IF7 | IKB | INHB | INPB | INPB | IKB | IKB | IKB | IKB | IKB |
| JUN | KAT6A | KDM5A | KDM5C | KDM6A | KDM6B | KDR | KEAP1 | KLF6 | KNAS |
| LCK | LREPB | MAP2K1 | MAP2K2 | MAP2K3 | MAP3K4 | MAPK1 | MAPK2 | MAPK3 | MCL1 |
| MDM2 | MDM4 | MELD1 | MELZ | MELZ | MELZ | MELZ | MELZ | MELZ | MELZ |
| MSH2 | MSH6 | MTHF | MYC | MYCL1 | MYCN | MYD88 | NBL | NFI |
| NF2 | NF2 | NF2BIA | NF2C-2 | NLRP3 | NOTCH1 | NOTCH2 | NPM1 | NRS5 | NTHL1 |
| NTRK1 | NTRK2 | NTRK3 | NTRK3 | NK3 | PALB2 | PARP1 | PARP2 | PARP2 | PARM1 |
| PDGFRB | PDGFB | PDGFRB | PDGFRB | PDGFRB | PDGFRB | PDGFRB | PDGFRB | PDGFRB | PDGFRB |
| POLD1 | POLD1 | POLD1 | POLD1 | POLD1 | POLD1 | POLD1 | POLD1 | POLD1 | POLD1 |
| PTCH1 | PTCH2 | PTEN | PTEN | PTEN | PTEN | PTEN | PTEN | PTEN | PTEN |
| RARA | RB1 | RET | RICTOR | RN4F | ROS1 | RPTOR | RUNK1 | SDHAF2 | SDHB |
| SDHC | SDHD | SDHD | SDHD | SDHD | SDHD | SDHD | SDHD | SDHD | SDHD |
| SMAD3 | SMAD4 | SMAD5 | SMACD4 | SMACD4 | SMACD4 | SMACD4 | SMACD4 | SMACD4 | SMACD4 |
| SPP1 | SRC | STAC | STAC | STAC | STAC | STAC | STAC | STAC | STAC |
| TGFBRI | TGRB | TGRB | TGRB | TGRB | TGRB | TGRB | TGRB | TGRB | TGRB |
| TSHR | TSHR | TSHR | TSHR | TSHR | TSHR | TSHR | TSHR | TSHR | TSHR |
| XCC3C | XCC4 | XCC5 | XCC6 | XCC6 | XCC6 | XCC6 | XCC6 | XCC6 | XCC6 |

4. Discussion
Several molecular profiling trials took place in recent years. Among those, Von Hoff et al. compared in 2010 the PFS2/PFS1 ratio, taking patients as their own controls, and considered there was a clinical benefit for a ratio ≥ 1.3. This study used a panel of genes for molecular testing. In this trial, the median PFS was 2.9 months and 27% of patients have PFS2/PFS1 ratio ≥ 1.3. Our study observed very similar results, but in contrast to this study we did not observe increased benefit of targeted therapies in breast cancer [9]. MOSCATO prospective trial also using a gene panel, showed a clinical benefit of the precision medicine strategy [12]. More than 1000 patients were included, with a new biopsy mandated by the protocol. Most tumor samples presented an actionable molecular alteration, and 199 patients were treated with a targeted therapy matched to a genomic alteration, mostly by inclusion in phase II/III trials. The PFS2/PFS1 ratio was > 1.3 in 33% of the patients. This study underlined objective
responses and improved overall survival with the matched treatment. Similarly, a US large-scale, prospective clinical sequencing initiative using MSK-IMPACT gene panel, performed matched tumor and normal sequences from a cohort of more than 10,000 patients with advanced cancer. 37% of patients harbored a clinically relevant alteration, and 11% were subsequently enrolled on a genomically matched clinical trial [1]. Interestingly, the top 3 mutated genes were also TP53 (41% of patients) KRAS (15%) and PIK3CA, consistent with our findings and confirming the role of these pathways in cancer biology. However, these promising results were not confirmed in a randomized trial. In particular, the SHIVA trial attributed randomly to patients with a molecular alterations detected using a gene panel, focused on three mains pathways (hormone receptor, PI3K/AKT/mTOR, RAF/MEK) a matched molecularly targeted agent or a treatment at physician’s choice [13]. No median PFS difference was highlighted similarly to what was found in our study.

In contrast to gene panels, WES is less used in precision medicine. In 2015, Beltran et al. enrolled prospectively 97 patients to analyze both tumor and normal tissue using WES [14]. WES provided...
For instance, a significant part of patients was heavily pretreated (60% of the patients had at least 3 lines of therapy), and some were not further treated despite available therapeutic propositions following NGS analysis due to physical impairment. Notably 33% of patients with disease progression and therapeutic proposal did not receive further therapy. These data support the conclusion that such strategy is not adapted to heavily pretreated patients with risk of rapid performance status deterioration. Likewise, in the recent I-PREDICT trial, patients were treated with combo-therapies of target agents. In this trial substantial numbers of patients dropped off from target therapies, mostly due to disease deterioration with hospice placement or demise [16]. Therefore, we believe that precision medicine approaches should be initiated earlier in the course of the disease. During study follow-up in our structure, we felt the need to adjust the molecular tumor board, proposing early patients’ inclusion and to stimulate earlier treatment with target therapies.

The recent I-PREDICT [16] and WINThER [17] trials invest the matching score strategies to improve patients selection. These studies raise the intuitive hypothesis that the more the therapy target mutations the more effective is the therapy. However, in EXOMA study of the matching score failed to demonstrate that patients treated with drugs that target more that 50% of targetable hits gain a benefit from this strategy. A major difference between I-PREDICT and EXOMA is that few patients in this trial received combo-therapies of target agents that may improve benefit from treatment.

The pitfall of exome sequencing is the absence of translocation and fusion detection. Despite this drawback, these results are similar to panel sequencing in terms of clinical efficacy. Probably RNA sequencing may be required in the future to assess fusion. In addition, such strategy could be helpful to take into account stromal microenvironment information and expression data [17]. In addition, determination of mutational signature should be implemented to help decision [18]. In conclusion, this trial contributes to assess the feasibility of tumor genomic profiling in routine care. However, patient treated with target therapies did not get clinical benefit from this strategy. The disappointing results of this study underline our superficial understanding of the mechanisms in cancer evolution and cancer heterogeneity. It also highlights the fact that even with the existing targeted agents, if our comprehension of the interactions in cancer cells, their evolution the interpatient and intra patient heterogeneity is not deeper, we may not improve current cancer treatment with precision medicine. In a technical point of view, the archived paraffin embedded tumors set a limit in the prospective nature of this trial because could they induce DNA fragmentation and mutations. Probably multiomics strategies and the incorporation of novel technologies like RNA-sequencing, whole genome sequencing and circulating cell-free DNA detection should be emphasized for future studies in order to estimate the possibility of novel targets and potential agents for these targets. Alternatively, exploration of particular “extraordinary response” or surprising failure of target therapies may help us to better understand cancer biology. All these efforts are important to improve Cancer Precision Medicine.

### Table 4
Therapeutic proposal.

| NGS-based proposals | N = 456 | N = 342 | N = 79 |
|---------------------|---------|---------|-------|
| Patients with therapeutic proposal after NGS analysis. n (%) | Yes 342 (75) | Yes 79 (23.1) | Yes 263 (76.9) |
| Treatment based on NGS analysis at disease progression. n (%) | No 114 (25) | No 263 (76.9) | No 9 (11.4) |

### Table 5
Summary of survival results. PFS: progression-free survival.

| Patients treated with non-NGS-based therapy | Patients with NGS based therapy | Patients with PFS2 and PFS1 data |
|--------------------------------------------|---------------------------------|---------------------------------|
| n = 89 | n = 48 | n = 137 |
| PFS of the 1st treatment - months | 3.6 [0.5 – 33.8] | 3.3 [0.5 – 10.1] | 3.5 [0.5 – 33.8] |
| PFS of the 2nd treatment - months | 2.1 [0.03 – 18] | 2.3 [0.02 – 10.1] | 2.2 [0.03 – 18] |
| Ratio 2nd PFS / 1st PFS | 0.6 [0.003 – 3.6] | 0.6 [0.1 – 6.1] | 0.6 [0.003 – 6.1] |
| Median [range] | 66 (74%) | 36 (75%) | 102 (74%) |
| ≤ 1.3 | 23 (26%) | 12 (25%) | 35 (26%) |
Declaration of competing interest

Other authors declare no relevant conflict of interests related to this publication.

Acknowledgments

We thank Isabel Gregoire for English editing.

Author contributions

FG designed the study, interpreted data and wrote the manuscript. MR collected data and wrote the manuscript. CR and JB performed statistical analysis, AB designed the methodology of the study, JN, JC, JDF collected clinical data, CT performed bioinformatics analysis, ID, SL, AH, LF, LB, JV, AH, JFGD, CL, PF, CB were the main investigators, JA performed analysis of constitutional mutations, LA validated and collected pathological samples, SN, LF performed genetic consultations, RB performed NGS analysis and somatic variant interpretation. All authors approved the final version of the manuscript.

Funding

This work was funding by the centre Georges Francois Leclerc. Funder had no role in study design, data collection, data analysis, interpretation, writing of the report.

Data sharing

Genomic data could be shared upon reasonable request to the corresponding authors in accordance to French law for genomic data.

Additional information

Correspondence and requests for materials should be addressed to Professor François Ghiringhelli MD, PhD, Department of Medical Oncology, Center Georges François Leclerc, 1 Rue du Professeur Marion, 21000 Dijon, France (e-mail: fghiringhelli@cgl.fr).

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2019.102624.

References

[1] Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, et al. Multifaceted landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017;23(6):703–13.
[2] Salvatore L, Aprile G, Arnoldi E, Aschle C, Carnaghi C, Cosimelli M, et al. Management of metastatic colorectal cancer patients: guidelines of the Italian Medical Oncology Association (AIOM). ESMO Open 2017;2(1):e000147.
[3] Kerr KM, Bubendorf L, Edelman MJ, Marchetti A, Mok T, Novello S, et al. Second ESMO consensus conference on lung cancer: pathology and molecular biomarkers for non-small-cell lung cancer. Ann Oncol 2014;25(9):1681–90.
[4] Dummer R, Hauschild A, Lindenblatt N, Pentheroudakis G, Kollhoff T. Cataract melanosomes: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2015;26(suppl 5):v126–32.
[5] Manolio TA, Rowley R, Williams MS, Roden D, Ginsburg GS, Buit C, et al. Opportunities, resources, and techniques for implementing genomics in clinical care. Lancet 2019;394(10197):511–20.
[6] Berger MF, Mardis ER. The emerging clinical relevance of genomics in cancer medicine. Nat Rev Clin Oncol 2018;15(6):333–65.
[7] Frampton GM, Fichtenholz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013;31(11):1023–31.
[8] Mateo J, Chakravarty D, Dienstmann R, Jezdic S, Gonzalez-Perez A, Lopez-Bigas N, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO scale for clinical actionability of molecular targets (ESCAT). Ann Oncol 2018;29(9):1895–902.
[9] Von Hoff DD, Stephenson JJ, Rosen P, Loesch DM, Borad MJ, Anthony S, et al. Pilot study using molecular profiling of patients’ tumors to find potential targets and select treatments for their refractory cancers. J Clin Oncol 2010;28(33):4877–83.
[10] Therasse P, Arluck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst. 2000;92(3):205–16.
[11] Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer. J Mol Diagn 2017;19(1):4–23.
[12] Massard C, Michiels S, Ferté C, Le Deley M-C, Lacroux L, Hollebecque A, et al. High-Throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the moscato 01 trial. Cancer Discov 2017;7(6):586–95.
[13] Le Tourneau C, Delord J-P, Gonzalves A, Gavoille C, Dubet C, Isambert N, et al. Moleculary 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(13):1324–34.
[14] Beltran H, Eng K, Mosquera JM, Sigaras A, Romanell A, Renne S, et al. Whole-Exome sequencing of metastatic cancer and biomarkers of treatment response. JAMA Oncol 2015;1(4):466.
[15] Zhang P, Lehmann BD, Shyr Y, Guo Y. The utilization of formalin fixed-paraffin-embedded specimens in high throughput genomic studies. Int J Genomics 2017;2017:1–9.
[16] Sicklick JK, Kato S, Okamura R, Schwaederle M, Hahn ME, Williams CB, et al. Molecular profiling of cancer patients enables personalized combination therapy: the i-predict study. Nat Med 2019;25(5):744–50.
[17] Rodon J, Soria J-C, Berger R, Miller WH, Rubin E, Kugel A, et al. Genomic and transcriptomic profiling expands precision cancer medicine: the winther trial. Nat Med 2019;25(5):751–8.
[18] Bertucci F, Ng CKY, Patounis A, Droin N, Piscuoglio S, Carubbia N, et al. Genomic characterization of metastatic breast cancers. Nature 2019;569(7757):360–4.