Usefulness and real-world outcomes of next generation sequencing testing in patients with cancer: an observational study on the impact of selection based on clinical judgement

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Summary

Background Next Generation Sequencing (NGS) panels are increasingly used in advanced patients with cancer to guide therapy. There is, however, controversy about when should these panels be used, and about their impact on the clinical course.

Methods In an observational study of 139 patients with cancer having an NGS test [from January 1st, 2017 to December 30th, 2020, in two hospitals (Hospital Universitario de La Princesa and Hospital Universitario Quironsalud Madrid) from Spain], we evaluated whether the clinical course (progression-free survival, PFS) was influenced by drug-based criteria [druggable alterations, receiving a recommended drug, having a favourable ESCAT category (ESMO Scale for Clinical Actionability of molecular Targets)] or clinical judgement criteria.

Findings In 111 of 139 cases that were successfully profiled, PFS was not significantly influenced by either having druggable alterations [median PFS for patients with druggable alterations was 170 (95% C.I.: 139–200) days compared to 299 (95% C.I.: 114–483) for those without; $p = 0.37$], receiving a proposed matching agent [median PFS for patients receiving a genomics-informed drug was 195 days (95% C.I.: 144–245), compared with 156 days for those that did not (95% C.I.: 85–226); $p = 0.50$], or having favourable ESCAT categories [median PFS for patients with ESCAT I-III was 183 days (95% C.I.: 104–261), compared with 180 (95% C.I.:144–215) for patients with ESCAT IV-X; $p = 0.87$]. In contrast, NGS testing performed within clinical judgement showed a significantly improved PFS [median PFS for patients that were profiled under the recommended scenarios was 319 days (95% C.I.: 0–658), compared to 123 days (95% C.I.: 89–156) in the non-recommended categories; $p = 0.0020$].

Interpretation According to our data, real-world outcomes after NGS testing provide evidence of the benefit of clinical judgement in patients with either advanced cancers that routinely need multiple genetic markers, patients with advanced rare cancers, or patients that are screened for molecular clinical trials. By contrast, NGS does not seem to be valuable when performed in cases with a poor PS, rapidly progressing cancer, short expected lifetime, or cases with no standard therapeutic options.

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Keywords: NGS panels; NGS testing; Precision medicine; Precision oncology; Targeted therapies; Real world outcomes; ESCAT; Clinical judgement

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Research in context

Evidence before this study

Next generation sequencing (NGS) is a novel genetic diagnostic technique that is increasingly ordered by cancer specialists, although there is controversy of whether upfront full molecular profiling is needed in all patients with cancer. We searched PubMed on December 21, 2022 using the terms "next generation sequencing" AND "cancer" AND "observational" and reviewed all publications of clinical studies. Only two observational study examined the real world testing of NGS, although limited to lung cancer. To date, no observational study has focused on the effect of NGS on survival of cancer patients.

Added value of this study

The aim of our observational study was to evaluate the impact of the clinical criteria with which a NGS test were ordered on the survival of patients with cancer. We have found that in four of the categories that we described for ordering NGS tests, progression-free survival (PFS) was significantly improved with respect to three categories (319 days versus 123 days). "Useful" categories for NGS testing represented 42% of cases while in 44%, NGS testing was "not useful". In an additional 14% of cases the test was "not necessary". In contrast to patient-derived characteristics, anticancer drug-based criteria (tumors showing druggable genetic alterations, patients receiving a recommended drug, or having a favourable ESCAT category) did not have a survival impact.

Implications of all the available evidence

We expect that our results will be of real clinical relevance for practicing clinicians that order NGS testing for patients with cancer, and could impact the indication guidelines for NGS that are issued by national and international agencies and scientific societies. In the future, categorised clinical judgement should be established before ordering NGS tests, and NGS testing should be performed preferentially in patients with either advanced cancers that routinely need multiple genetic markers, patients with advanced rare cancers, or patients that are screened for molecular clinical trials.

Introduction

Cancer genomic profiling in patients has opened new horizons in investigational oncology. In a first wave of genetic studies, associations of gene mutations were established with the efficacy of some drugs, usually small molecules with kinase inhibitor properties. Gene alterations were usually highly penetrant mutations in a protein kinase-encoding gene that causes its hyperactivation or gain of function.[1–5] Cases in which a loss-of-function event results from deleterious mutations in genes such as BRCA1/2 or PTEN (which are highly responsive to PARP inhibitors[6] or PI3K inhibitors,[7] respectively), also showed associations linking a genomic profiling result to therapy allocation. A second wave of discoveries linked more complex genomic alterations to specific drug allocations. Examples were the use of immunotherapies in patients with high tumour mutational burden (TMB),[8] PARP inhibitors or platinum compounds in patients with genome-wide traits of DNA damage repair (DDR) impairment,[9–12] or those associations of a specific gain-of-function or loss-of-function event with high sensitivity to an agent that targets a pathway different to that in where the mutations lie.[13] Currently, a third wave of studies is trying to find associations between genomes lacking alterations of the former types and response to specific drugs or drug combinations. This task is proving particularly difficult, and prospective trials of different design (basket, umbrella or algorithm-testing), relying on different genomic material and techniques (DNA WES, gene panels, RNA-seq) and approaches (liquid or solid biopsies) have so far achieved modest response rates for drugs administered outside their FDA-approved indication.[14–28]

Given the increasing number of potential targets for cancer therapies along the tumour genome, novel applications have been developed that provide information about them with the aim to guide therapeutic selection. Next generation sequencing (NGS) is a gene sequencing technology that offers very high throughput, scalability and speed, allowing sequencing of whole cancer genomes (whole genome sequencing or WGS) from dozens to hundreds of patients within a few days. However, for clinical applications, most of the genomic information is not required for target identification (albeit useful for research purposes), and smaller scale applications have been developed. Most often, in the clinical setting, whole-exome sequencing (WES) or simply sequencing a number of "cancer-relevant" genes (ranging from 50 to approximately 500; "targeted NGS panels) are sufficient for target identification, providing a significant cost advantage relatively to WGS. In the real-world clinical practice, NGS panels are being increasingly ordered by cancer specialists, although the controversy of whether upfront full molecular profiling is needed in all patients with cancer is not a solved issue, as a recent contraposition of views shows.[29] Initial studies studying NGS panels in advanced cancers such as the MOSCATO or the SHIVA trials reported a 5% actual disease control rate among all patients in whom profiling was attempted.[15,28] More recently, a real-world study of the clinical application of NGS in advanced breast cancer showed that NGS testing allowed molecular-guided therapy in only 4.7% of patients.[30]

The poor overall efficacy results of these studies was related to either poor quality samples, the absence of detectable druggable alterations (as per protocol definition), or the inability of delivering the matching drug when there was a druggable alteration[31] (a druggable alteration refers to a biological target, such as a protein encoded by a mutant, amplified or fusion gene product present in cancer cells, that is known or predicted to bind with high affinity to a given drug, where, by definition, the binding of the drug to the target alters its function, but may or may not lead to a therapeutic benefit for the patient). Other studies have observed that the efficacy of targeted agents, even in the presence of the putative druggable target may be low outside their agency-approved indication.[32,33] Furthermore, some common alterations, such as PIK3CA mutations, can modulate the response to the target inhibition likely due to co-existing alterations such as FGFR, MYC, GATA1 or TP53.[34–36] Finally, it has also been described that, in the absence of oncogenic-addiction driver mutations, tumours behave in a polyclonal manner, and a decision on an agent against a detected target may result in the proliferation of disease subclones that will cause disease failure.[37] Because of these limitations, it is of the utmost relevance that the situations in where NGS testing may prove useful are narrowed down.

There have been some academic attempts to characterise how and when to use NGS testing in patients with cancer. In 2018, the European Society for Medical Oncology (ESMO) published the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT), which provided a systematic framework to rank molecular targets based on the evidence that is available supporting their value as clinical targets.[38] ESCAT divides molecular alteration-drug match pairs in 6 categories: I: alteration associated with improved outcome in clinical trials, making the target suitable for routine use and recommend specific drug when detected; II: alteration associated with antitumour activity but of unknown magnitude, making the target likely a biomarker for a specific patient population but additional data are needed; III: the alteration is suspected to improve outcome based on clinical data in other tumour type(s) or with similar alterations, but no evidence exist for that particular tumour/alteration; IV: the evidence of activity is limited to the preclinical setting; V: evidence of activity exists, but is not associated with clinically meaningful benefit, supporting the study of co-targeting approaches; and X: lack of evidence of actionability.[38] A recent review has highlighted some limitations and implementation challenges of this molecularly-guided treatment strategy.[39] In 2020, we published a patient-oriented NGS recommendations, that defined tentatively when genomic profiling could be useful and therefore recommended in several patient categories. We proposed that NGS indication should focus on either advanced cancers with multiple molecular markers that are relevant for initial therapy -such as advanced NSCLC, colorectal cancer or melanoma-, advanced rare cancers, exceptional responders or situations when a clinical trial with molecular screening is available.[40] We did not recommend the performance of a NGS test in patients with rapidly progressing tumours or with either poor performance status or short expected lifetime since, we argued, the test turnaround time or the treatment tolerance would impede administering a genomic-informed treatment, even in the case of a routine use suggestion.

Our current study has appraised whether the use in a real-word setting of either molecular target-based or clinical judgement-based recommendations have an influence on the clinical course of patients. We report that NGS testing in advanced cancer may provide some clinical benefit. While factors such as harboring a druggable alteration or having ESCAT categories had a limited impact, adhering to clinical recommendations provided a significant benefit in outcomes. We have also identified two additional clinical categories that may lead to ordering a genomic panel and were not previously described in our previous set of guidelines, and we have incorporated them in our revised classification.

Methods

Study population

Eligible cases for this retrospective observational study were those in whom a commercial tumour genomic profiling test was ordered. No limitations were established for tumour type, treatment line, metastatic sites, organ function or ECOG performance status. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice standard. The research protocol was approved by the IRB of the Hospital Universitario La Princesa (# 4444/21).

All patients were tested with the FoundationOne CDx test, which sequences 324 genes from a solid tumour tissue sample and issues a report listing the detected alterations (mutations, amplifications, indels or fusions, as well as tumour mutational burden or LOH) plus a number of suggested therapies (if any) against each. After patients signed the Informed Consent Form, cases were recorded in a database of genomic profiling, and full medical records were reviewed.

We selected progression-free survival as the clinical outcome because it reflects reliably the effect of the first therapy given after testing. Recently, it has been shown that real-world PFS correlates well with conventional clinical trial PFS.[41]

Descriptive parameters (demographics, clinical characteristics, reasons for ordering the test) are given for all patients (n = 139). Self-reported sex was used to determine patients' sex. In patients with successful profiling (n = 111), we established drug-based

characteristics such as drug treatment allocation analysis, presence of druggable alterations, molecular landscape, and actual delivery of a recommended drug, ESCAT categories,[38] and reasons for allocation or not to the suggested therapies, as well as clinical characteristics such as our NGS Indications Categories.

Statistical analysis

Patient categories (according to the reason that motivated clinicians to order an NGS test) were allocated according to clinical judgement. We compared whether patients in some categories had better clinical outcomes than others, in an attempt to support (or not) our classification. In order to do this, the outcome analysed was progression-free survival (PFS) time, calculated from the reception of the profiling results. PFS estimates were compared with Kaplan-Meier curves and the Log-Rank test among different subgroups. Although testing the effect of performing an NGS test in overall survival (OS) was not the primary outcome of the study, given the positive associations observed for PFS, we decided to compare the effects in three different scenarios: whether receiving or not a matching drug for those patients with at least one reported druggable alteration, belonging to different clinical categories, or adhering or not to the "indicated categories" was associated with differences in overall survival. Thus, these were post-hoc, non-planned OS analyses. For all OS comparisons, time was calculated from the reception of the profiling results until death or censoring. All percentages reported along the manuscript and tables/figures were rounded to the nearest integer. All statistical tests run with the SPSS V. 19 software package.

Role of the funding source

Funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. All authors had access to the full dataset; MQF and RC decided the submission for publication with the agreement of all co-authors.

Results

Patients, molecular targets and treatments

We analysed 139 patients in the period January 1st, 2017–December 30th, 2020, in two hospitals (Hospital Universitario de La Princesa and Hospital Universitario Quironsalud Madrid) in Spain. Their clinical and demographic characteristics are shown in Supplementary Table S1. Molecular testing was ordered a median of 246 days (range: 1–4113; interquartile range: 72–696) after the diagnosis of incurable disease. The median number of treatment lines for advanced disease prior to ordering molecular testing was 1, ranging from 0 to 7 (interquartile range: 1–3) (Supplementary Table S1). Median follow-up was 333 days from the moment of ordering the NGS molecular profiling (range: 0–1852; interquartile range: 119–834).

A CONSORT patient flow diagram is shown in Fig. 1. Successful profiling was obtained in 111 cases. The number of patients not receiving a valid result due to poor sample or poor DNA quality was 28 (21%); most

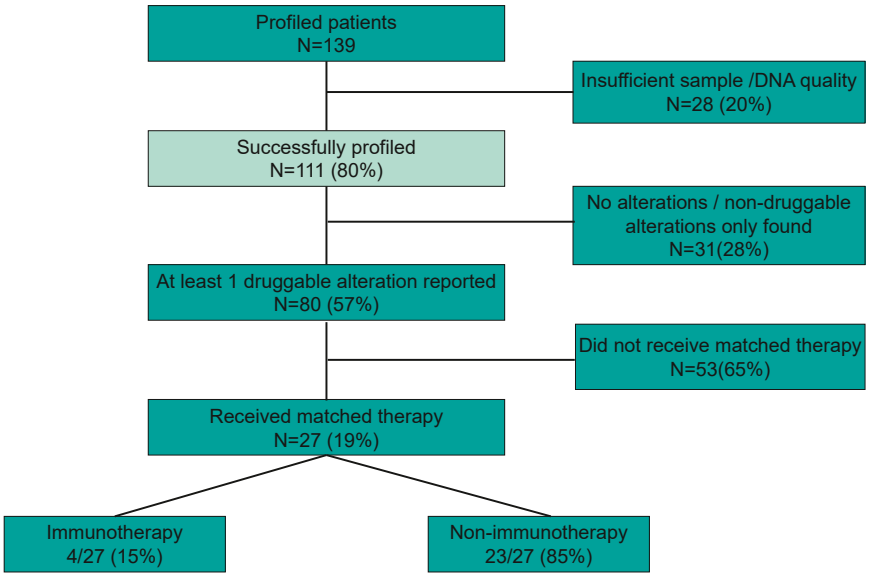


Fig. 1: **Patient flow diagram.** This diagram depicts how many patients and for which reason did or did not reach treatment allocation. The percentages on the up-to-down flow are shown relatively to the total number of initial patients (n = 139), whereas each of the boxes in the right-hand side shows the percentage of patients lost relative to the previous step, except when indicated.

of these biopsies were either >10 year-old [archival biopsies from the primary tumour; n = 12] or bony lesion biopsies (n = 7).

The median number of reported genomic alterations in successfully profiled patients was 3 (range 0–16; interquartile range: 2–5). The most frequent alterations are shown in Fig. 2. Of the 426 reported alterations, 139 were reported as "druggable" (median per patient = 1; range: 0–6; interquartile range: 0–2) and 277 as "non-druggable" (median per patient = 2; range: 0–11; interquartile range: 1–3).

In the 111 patients successfully profiled, a potentially druggable alteration was identified in 80 cases (72%). Twenty-seven (24%) eventually received a drug that was theoretically suitable for one of their druggable alterations. The majority of the allocated therapies were small molecules, and in 4 cases the allocated drug was an immune checkpoint inhibitor. Fifty-three patients were successfully profiled and had druggable alterations but received other therapies (48%), mainly chemotherapy and hormonal therapy. Thirty-one patients (28%) had either undruggable alterations or no alterations detected, and these received chemotherapy, hormonal therapy or best supportive care. The allocated drugs, and the intention (i.e., against which target) with which they were used for the successfully profiled patients are listed in Table 1. The median PFS time of the 111 successfully profiled patients was 170 days (95% C.I.: 137–202).

Impact of drug target-based categories: druggable targets, allocation to genomic profiling-informed treatment, and ESCAT categories

We first compared the PFS of the patients that had a reported druggable alteration (n = 80) with those that did not (n = 31), since it has been suggested that patients with druggable alterations might have an intrinsically better disease prognosis compared to those in whom no druggable alterations are found, regardless of receiving or not the suggested drug.[42] Median PFS in patients with druggable alterations was 170 days (95% C.I.: 139–200), compared to 299 days (95% C.I.: 114–483) for patients without. The numerically improved PFS in patients with no druggable alterations, however, did not reach statistical significance ($p = 0.37$; Fig. 3A). PFS did not change significantly according to the number of druggable alterations (Supplementary Fig. S1).

We next evaluated the outcome in patients had at least one alteration reported as druggable, comparing patients that eventually received a genomic profiling-informed drug therapy (n = 27) versus those that did not (n = 53). Patients receiving the suggested drug had a median PFS of 195 days (95% C.I.: 144–245) compared with 156 days (95% C.I.: 85–226) for patients that did not receive the suggested drug. This difference was not significant ($p = 0.50$; Fig. 3B).

We identified clinical characteristics that might explain not receiving the drug or drugs that were suggested by genomic profiling and classified in 10

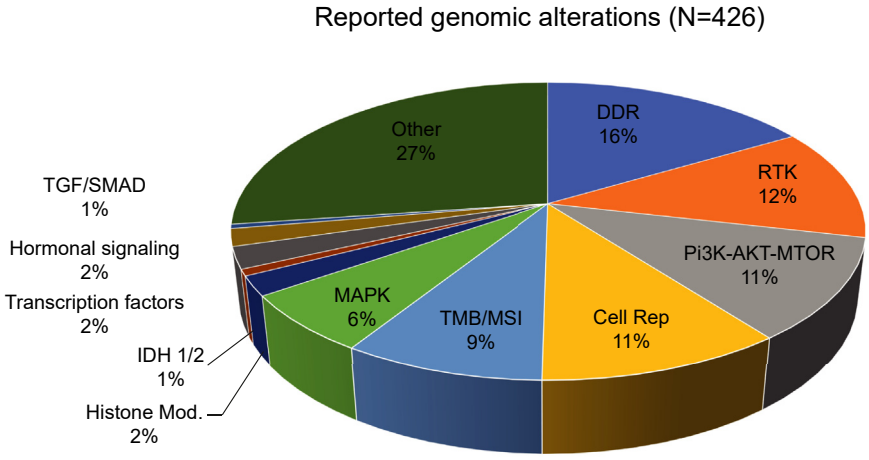


***Fig. 2:* Reported genomic alterations in successfully profiled patients.** The most frequent (druggable or non-druggable) alterations were present in genes encoding for proteins of the DNA Damage Repair pathway (DDR) such as ATM, ATR, BRCA1/2, ARID1A or BRIP1; Receptor Tyrosine Kinases (RTKs) such as EGFR, ERBB2 or FGFR1-3; Pi3K-AKT-MTOR pathway; cell replication (Cell Rep) pathway (gain-of-function mutations in CDKs or loss-of-function of P27, RB, or other cell cycle checkpoints), or MAP-Kinase pathway (MAPK). In addition, profiling test also reported as druggable alterations the presence of high or intermediate tumour mutational burden (TMB) and/or presence of microsatellite instability (MSI) secondary to DNA mismatch repair. Other less frequently mutated genes were histone modifiers (KMT2C, EZH2, SETD2), transcription factors (MYC, GATA), SMAD proteins, IDH1/2, or genes implicated in hormonal signaling (ESR1 mutation, AR amplification, or TMPRSS2 fusions). Twenty-seven per cent of the alterations could not be classified in any of the former (typically targetable) pathways.

Patient subgroup	Treatment administered	N (%)
Druggable alterations detected, allocated to a matching therapy (N = 27)	Immune checkpoint inhibitors (intermediate or high tumor mutational burden)	4 (15%)
	Everolimus (3 cases as an inhibitor of Pi3K-AKT-MTOR pathway; 1 as FBXW7 inhibitor)	4 (15%)
	Enzalutamide plus talazoparib (prostate cancer with DDR defects)[a]	4 (15%)
	Pazopanib (as FGFR1/2 inhibitor)	3 (11%)
	Crizotinib (1 case as ALK inhibitor; 1 case as MET inhibitor)	2 (7%)
	Erlotinib (as EGFR inhibitor)	2 (7%)
	Dabrafenib-trametinib (for NF1 loss)	1 (4%)
	Vandetanib (as RET inhibitor)	1 (4%)
	Encorafenib-binimetinib-cetuximab (for a BRAF mutation)	1 (4%)
	TDM1 (HER2 inhibitor, case with a ERBB2 activating mutation)	1 (4%)
	Pertuzumab-trastuzumab (HER2 inhibitor, case with ERBB2 amplification)	1 (4%)
	Olaparib (for a BRCA2 mutation)	1 (4%)
	Neratinib (for an ERBB2 activating mutation)	1 (4%)
	None[b]	1 (4%)
Druggable alterations detected, but not allocated to a matching therapy (N = 53)	Chemotherapy	13 (24%)
	Best supportive care only	12 (23%)
	Hormonal blockade	6 (11%)
	Chemotherapy plus targeted therapy	2 (4%)
	Immunotherapy	1 (2%)
	Targeted agent (olaparib)	1 (2%)
	Watch-and-wait decision (inflammatory myofibroblastic tumor)	1 (2%)
	Not required[c]	8 (15%)
	Unknown[d]	9 (17.0%)
No alterations or non-druggable alterations detected (N = 31)	Chemotherapy	14 (45%)
	Hormonal blockade	8 (26%)
	Best supportive care only	7 (23%)
	Immunotherapy	1 (3%)
	None[e]	1 (3%)

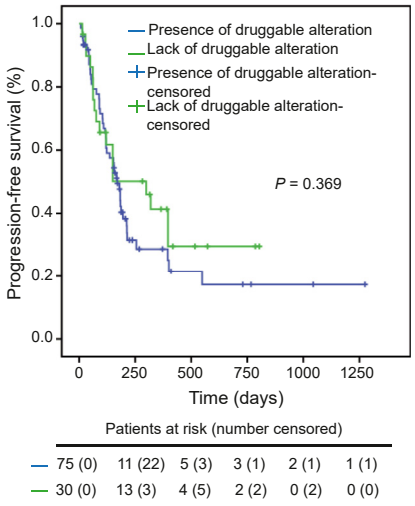
[a]Patients with prostate cancer and a detected alteration in the DNA Damage Repair (DDR) system were allocated to a clinical trial in where patients received enzalutamide and talazoparib, the latter in a double-blinded, placebo-controlled manner. [b]At the moment of this report, the patient is still in response to the first-line treatment (lung adenocarcinoma in response to carboplatin, pemetrexed and nivolumab). [c]With the exception of 1 patient, seven of these patients were profiled while still in complete response to first line treatment or after resection of a primary tumor with curative intention; thus, no treatment had been allocated at the moment of the present analysis. The last patient had a resection of a single-lesion metastatic relapse of a lung cancer, and thus was considered as well in complete response. [d]These patients were either lost to follow-up or were enrolled in clinical trials in other institutions for whom we were unable to retrieve the information. [e]This patient was profiled while in complete response to first-line immunotherapy.

Table 1: **Drug allocation in successfully profiled patients (N = 111).**

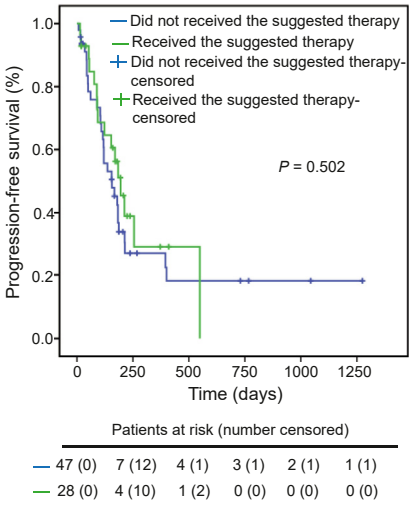
categories: (a) lack of identified targets; (b) identified but informed as undruggable by the profiling report; (c) identified targets but of low ESCAT; (d) identified targets of ESCAT I-III but lack of ability to prescribe it for the patient's tumour; (e) identified target but the patient was enrolled in a clinical trial in which he/she received a different agent/s; or (f) patient in advanced condition precluded receiving the suggested treatment/s. Additional reasons were either (g) not having progressed to the previous treatment line yet, (h) having received a treatment for a primary tumour with curative intention, (i) having received already the suggested drug, or (j) being lost-to-follow-up. The distribution of patients is shown in Fig. 3C. The most common reasons for not receiving a profiling-informed drug among the 84 that did not receive a drug informed by profiling were the lack of druggability of the alterations found (9%), the inability to finance the prescription of the informed drug (17%), or the rapid deterioration of the patient (17%).

Concerning ESCAT, the categories established for each successfully profiled patient is shown in Supplementary Table S1. When the PFS time was compared between the patients that were allocated to the best ESCAT possibility and the patients that were not allocated to a genomic profiling-informed drug, the comparison did not yield statistically significant results: the median PFS time was 195 days for the former (95% C.I.: 144–245), compared to 156 days (95% C.I.: 116–195) for the latter (p = 0.80; Fig. 3D). Similarly, patients in whom a high ESCAT (I-III) was suggested, showed a virtually identical outcomes to those with ESCAT IV to X [183 (95% C.I.: 104–261) versus 180 (95% C.I.: 144–215) days; p = 0.88; Fig. 3E].

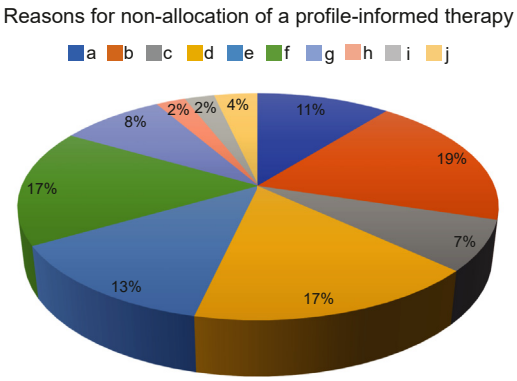
A


Presence of druggable alteration
Lack of druggable alteration
Presence of druggable alteration-censored
Lack of druggable alteration-censored
P = 0.369
Progression-free survival (%)
1.0
0.8
0.6
0.4
0.2
0.0
0
250
500
750
1000
1250
Time (days)
Patients at risk (number censored)
75 (0) 11 (22) 5 (3) 3 (1) 2 (1) 1 (1)
30 (0) 13 (3) 4 (5) 2 (2) 0 (2) 0 (0)

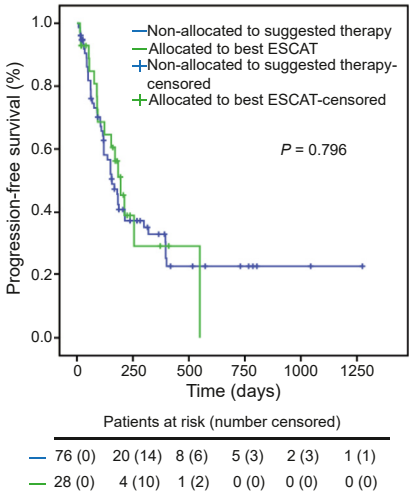
B


Did not received the suggested therapy
Received the suggested therapy
Did not received the suggested therapy-censored
Received the suggested therapy-censored
P = 0.502
Progression-free survival (%)
1.0
0.8
0.6
0.4
0.2
0.0
0
250
500
750
1000
1250
Time (days)
Patients at risk (number censored)
47 (0) 7 (12) 4 (1) 3 (1) 2 (1) 1 (1)
28 (0) 4 (10) 1 (2) 0 (0) 0 (0) 0 (0)

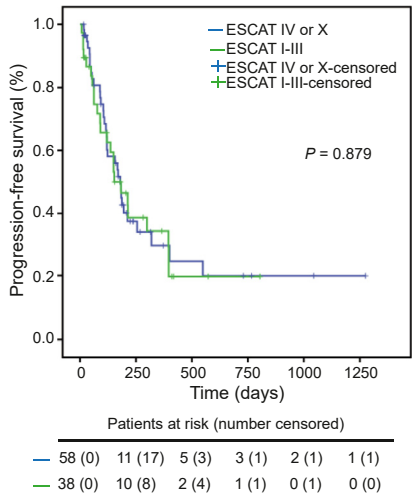
C


Reasons for non-allocation of a profile-informed therapy
a b c d e f g h i j
11%
19%
7%
17%
13%
17%
8%
2%
2%
4%

D


Non-allocated to suggested therapy
Allocated to best ESCAT
Non-allocated to suggested therapy-censored
Allocated to best ESCAT-censored
P = 0.796
Progression-free survival (%)
1.0
0.8
0.6
0.4
0.2
0.0
0
250
500
750
1000
1250
Time (days)
Patients at risk (number censored)
76 (0) 20 (14) 8 (6) 5 (3) 2 (3) 1 (1)
28 (0) 4 (10) 1 (2) 0 (0) 0 (0) 0 (0)

E


ESCAT IV or X
ESCAT I-III
ESCAT IV or X-censored
ESCAT I-III-censored
P = 0.879
Progression-free survival (%)
1.0
0.8
0.6
0.4
0.2
0.0
0
250
500
750
1000
1250
Time (days)
Patients at risk (number censored)
58 (0) 11 (17) 5 (3) 3 (1) 2 (1) 1 (1)
38 (0) 10 (8) 2 (4) 1 (1) 0 (1) 0 (0)

Impact of clinical judgement categories

We appraised whether in our clinical practice, cancer specialists followed, and to what extent, our published clinical judgement categories recommendations. We used a similar layout that we used previously,[40] with some variations. First, the categories poor PS and short expected lifetime were fused (n = 20), because were indistinguishable clinically. Second, we identified two additional situations that we had not anticipated in our first appraisal: patients with advanced malignancy that had a good performance status (ECOG 0-2) but do not have standard therapeutic options and do not desire or are not eligible for a clinical trial (category 5; n = 39), and patients with advanced malignancy of any type for whom there are well established standard non-molecular options (category 9; n = 15).

In order to test to what extent our recommendations optimise the use of gene panel testing, we compared the PFS times of patients in whom, according to our criteria, NGS profiling was recommended ("useful"; categories 1–4) versus those in whom it was not ("not useful"; categories 5–7), excluding from this first analysis those in whom NGS profiling was deemed simply "not necessary" (categories 8 and 9). The 59 patients from categories 1–4 (recommended) showed a statistically significantly higher PFS than those patients with categories 6–7 (n = 22) in whom the testing was not recommended according to our previous guidelines [319 (95% C.I.: 0–658) versus 116 (95% C.I.: 0–241) days; $p < 0.001$; Fig. 4B]. Category 5 was a novel addition, only identified in the current patient series, and deemed in principle "not recommended" according to clinical judgement. We performed a separate outcome analysis of this category and found that their prognosis was adverse, with a median PFS (150 days; 95% C.I.: 74–226) significantly poorer than patients in categories 1–4 ($p = 0.038$; Fig. 4B) although higher than those in categories 6–7 ($p = 0.018$; Fig. 4B). Thus, this preliminary analysis seems to support the placing of category 5 as shown in Fig. 4A, along categories 6 and 7.

To complete the outcomes evaluation, we compared the other new category (9) with categories 1–4. The comparison favored the "recommended" group although with nonstatistical significance [319 (95% C.I.: 0–658) versus 183 (95% C.I.: 111–254) days; $p = 0.13$; Supplementary Fig. S2]. When we pooled together all the non-indicated categories (5, 6, 7, 8 and 9; of note category 8 includes only early-stage cancers, and 9 includes many patients in first-line metastatic setting), and compared their PFS to the indicated categories (1–4). The comparison still favored the "useful" categories [319 (95% C.I.: 0–658) versus 123 (95% C.I.: 89–156) days; $p = 0.0020$; Fig. 4C].

Therefore, in our revised classification (Figs. 4A), 59 of the patients analysed (42%) had a NGS test that was considered "useful", in comparison with 19 patients (14%) considered "not necessary", and 61 (44%) considered "not useful". New categories (5 and 9) were allocated to the not-useful and not-necessary groups, respectively, according to clinical judgement; PFS analysis supports this allocation.

We also conducted a preliminary evaluation of the impacts in OS. In case of having a defined druggable target, it is possible that these patients did not receive the suggested option in the immediately subsequent line, but the attending oncologist might decide to administer it in later lines because of different reasons (for example, availability of other standards, low ESCAT, or simply that the drug became available for prescription months or years after having the NGS report). Such reasons may not impact in the PFS, but they may translate into a long-term impact in OS. Thus, among patients with at least one reported druggable target, we compared the OS between those that received or not the matching drug; median OS were 593 days (95% C.I.: 206–979) versus 936 days (95% C.I.: 548–1323), respectively ($p = 0.54$; Fig. 5A). We also tested whether the indication categories impacted in global OS, replicating the analysis of the category groups shown in Fig. 4B and C. Fig. 5B shows the median OS comparison between categories 1–4 (group 1), 5 (group 2), and 6–7 (group 3). Median OS were not reached, 593 (95% C.I.: 185–1001) and 136 (95% C.I.:59–213) days, respectively; OS favored group 1 over 3 ($p < 0.001$) and with borderline statistical significance over group 2 ($p = 0.060$). Fig. 5C shows the OS comparison between

Fig. 3: **ESCAT categories, drug allocation and impact of genomic profiling-informed drug allocation. (A)** Progression-free survival chart of patients with or without a reported druggable alteration in their genomic profiling. **(B)** Within patients in whom a druggable target was reported (n = 80), the progression-free survival of patients that finally received, or not, the suggested therapy is shown. **(C)** Chart showing the percentage of patients (n = 84) that did not have a genomic profiling-informed drug allocation because of each of the 10 identified possible reasons: (a) lack of identified targets; (b) identified but informed as undruggable by the profiling report; (c) identified targets but of low ESCAT; (d) identified targets of ESCAT I-III but lack of ability to prescribe it for the patient's tumour; (e) identified target but the patient was enrolled in a clinical trial in which he/she received a different agent/s; or (f) patient in advanced condition precluded receiving the suggested treatment/s. Additional reasons were either (g) not having progressed to the previous treatment line yet, (h) having received a treatment for a primary tumour with curative intention, (i) having received already the suggested drug, or (j) being lost-to-follow-up. **(D)** Kaplan-Meier curve showing the progression-free survival comparison between patients that were allocated a drug according to their best ESCAT category and those that did not receive the therapy suggested by the genomic profiling. (**E**) Kaplan Meier curves comparing PFS of patients with high ESCAT (I-III) and low ESCAT (IV and X).

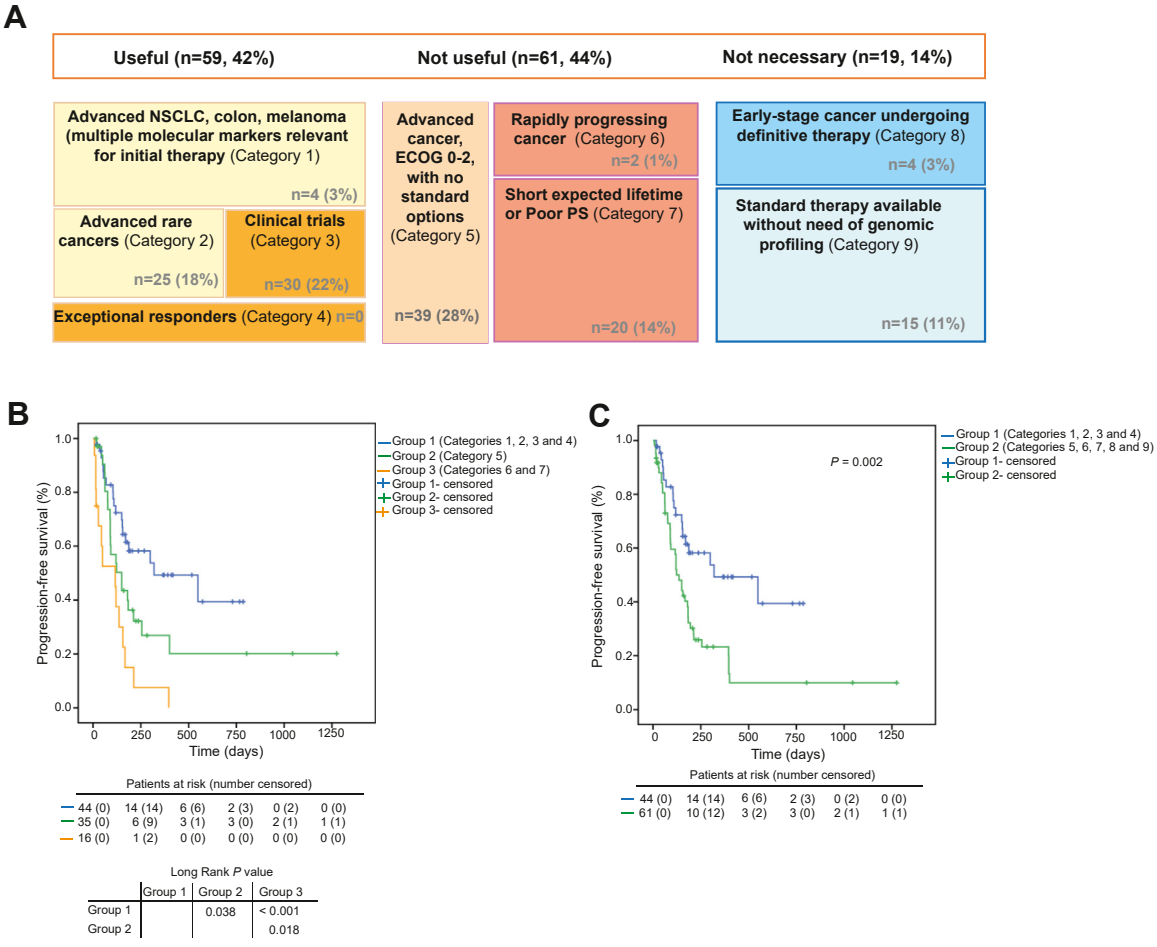


***Fig. 4:* Categorisation of genomic profiling test ordering and impact in PFS. (A)** The diagram depicts the percentage of patients (n = 139) in whom the test was ordered according to the previously identified indications,[37] plus two previously unidentified situations (categories 5 and 9). These have been categorised as "not necessary" and "not useful", respectively, due to the PFS results that are shown in 4B. Patients that had an ECOG 3–4 also were in the "Short expected lifetime" category (<12 weeks) and were grouped together in category 7. **(B)** Kaplan-Meier PFS curve of advanced patients with cancer with NGS testing indication (categories 1–4) versus patients in the "not useful" categories (6 and 7) or the new "not useful" category 5. **(C)** Kaplan-Meier PFS curve of advanced patients with cancer with NGS testing categorised as "useful" (categories 1–4) versus "not useful" or "not indicated" (categories 5–9).

the "indicated categories" (1–4) and the rest (5–9); the comparison was statistically significant (p = 0.0040) favoring the former group: median OS not reached versus 401 days (95% C.I.: 85–717).

Discussion

The ability to sequence many genes in a short period of time, and the success in associating genomic alterations with drug indications, have led to the proliferation of NGS tests for cancer. However, the actual impact of NGS testing in cancer has some limitations. NGS use, while logically focusing on gene alterations, has not taken into account the impact of clinical characteristics such as the general health of patients on the test-associated outcomes. Similarly, a benefit of NGS has been evaluated for cost-effectiveness when compared with single-gene testing,[43] but an NGS-derived survival improvement has not been clearly addressed. Finally, the design of NGS clinical trials or NGS real-world studies reflect the difficulties that have been described previously in reliably evaluating new diagnostic tools.[44] For an adequate routine rationalisation of this resource, comprehensive recommendations and guidelines are clearly needed.

In 2020, we published a set of general recommendations for NGS genomic testing in patients with advanced cancer, based on their clinical characteristics.[40] Some of the recommendations included those situations in which one-at-a-time testing for all the known druggable drivers might be both impractical due to diagnostic sample exhaustion and have higher cost

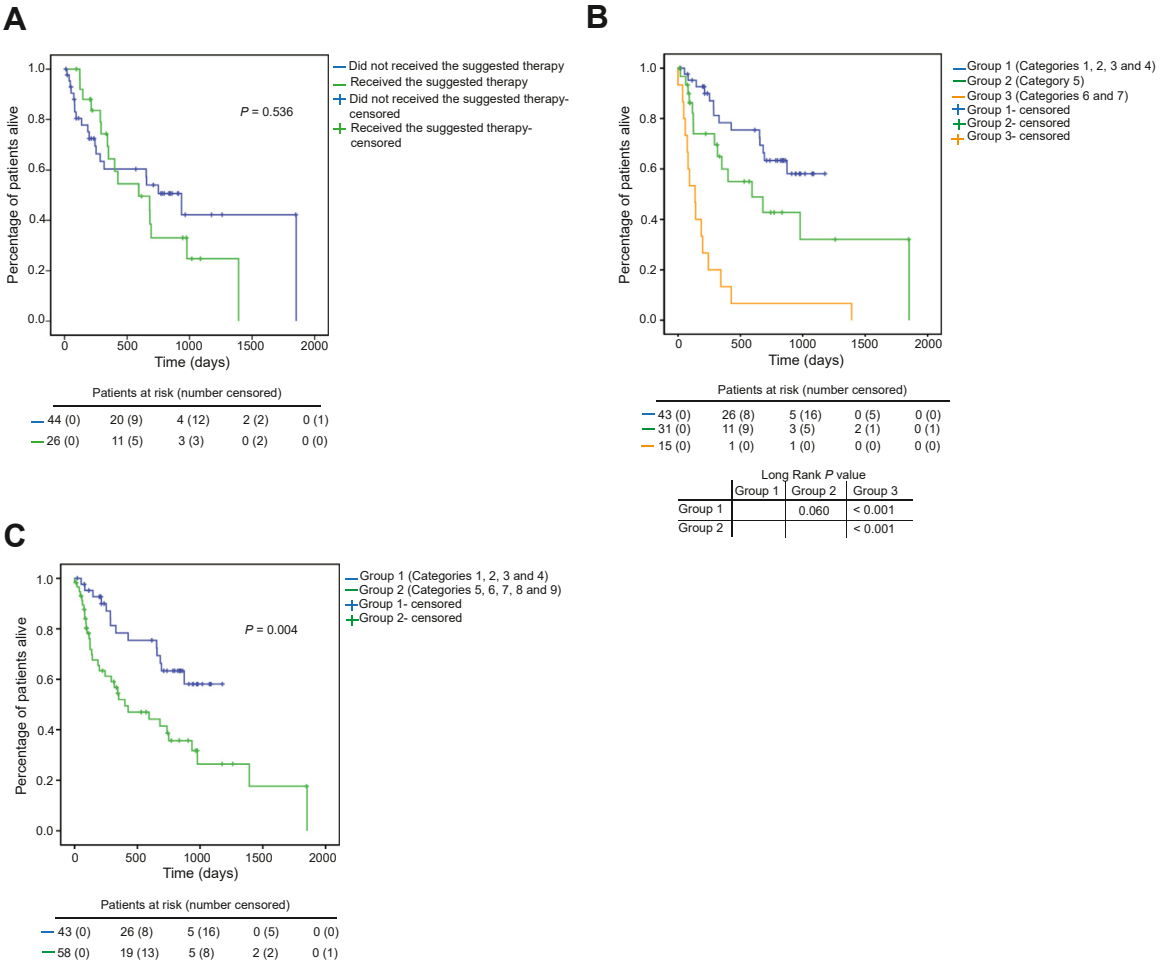


Fig. 5: **Effects of NGS profiling in Overall Survival. (A)** Kaplan-Meier OS curve for patients with successful profiling and at least one suggested druggable target that were or were not treated with a matching drug accordingly. **(B)** Kaplan-Meier OS curves for patients in categories 1–4, 5 or 6–7. **(C)** Kaplan-Meier OS curves for patients with indication of NGS profiling according to our recommendations (categories 1–4) versus the remaining (5–9).

compared to a single NGS test, as in first-line metastatic lung cancer; some advanced rare cancers early in the course of the disease; patient screening for inclusion in clinical trials of molecularly-guided therapy; or characterising the genotype of unusual responders. Using a different approach, the European Society for Medical Oncology categorised patients according to their "druggable" genomic alterations according to the degree of evidence supporting the use of each mutation-drug pair (ESCAT).[38]

In order to gather actual evidence about the clinical validity of either drug target-based or patient-based classifications, we performed a real-world observational study with patient follow-up that addressed whether categorisations for NGS testing had an impact on the clinical course of patients.

In our series, 80% of the patients had a valid genomic profile with centralised testing, a figure somewhat higher than that reported in clinical trials[15,17–19,28] although similar to other real-world series.[45–50] The nature of the reported genomic alterations was also in line with those reported in other series.[39–41,43–45] Centralised NGS testing, which is common in standard practice in hospitals that have not set their own NGS facility and/or analytic pipeline, has some advantages such as standardisation, frequent technological and bioinformatic update, and scale economy. However, we perceive three limitations in centralised testing for real-world practice. First, the astringent sample-quality standards and fixed protocols may make that some samples –in which a more detailed, personalised or customised DNA-extraction protocol could have yielded a valid NGS run– are never analysed (for example, prostate cancer samples from bony lesions, where de-calcification protocols and limited cellularity can hamper DNA amount and quality

yields). Second, centralised testing leads to approximately 6-weeks delay in the clinical decision (whether to "try again" or simply prescribe a different therapy). A dialogue with the pathologist, technician or bioinformatician regarding specific problematic samples is easier and quicker in a local environment, possibly increasing the percentage of valid samples. The third limitation is that centralised NGS vendors do not have access to granular clinical data, and clinicians do not have access to full NGS data: this complicates greatly the conduction of large-scale genomics research, where thousands of clinical cases remain non-analysed in detail. On the other hand, academic hospitals with their own set up procedures for NGS can solve better (and faster) individual sample uses and conduct tertiary analysis research often, although with limited number of patients. Disadvantages in these academic settings are limited funding, difficulties in updating technology and non-standardised sequencing protocols, panels and/or analytic pipelines between different institutions. Recent intermediate solutions, such as "regional molecular tumour boards",[51] development of standardised protocols and analytic tools as "kits" for local use,[52] or even country-wide (academic) initiatives[51,53] may implement the best of both worlds and contribute to protocol/pipelines standardisation, technological update, and tertiary analysis approaches. Alternatively, others have addressed sample-related limitations or availability by proceeding with cell-free DNA. Blood cell-free DNA contains fragments of tumour DNA and may represent a broad assessment of different tumour clones from different lesions, and allow recovering tumour DNA in cases where metastases are difficult to sample or simply there are not available samples with a simple blood extraction.[54,55] A recent landmark trial demonstrates the feasibility of this approach, but not all tumours shed sufficient DNA to the blood so that it is detectable (regardless of the tumour burden).[17] Sensitivity can be improved by increasing sequencing depth when we narrow-down the genes that we aim to capture to a small panel or just a few known hot-spots or mutations (with digital PCR), but this would be useful only for tumours with known drivers (i.e., EGFR-mutant lung cancer) or mutations (a tumour where we have already sequenced a solid lesion), thus, not being a technique aimed at target finding but more probably at disease burden monitor in this case.[54,55] Thus, the ideal technique for searching for a broad number of druggable alterations probably requires a case-by-case assessment in the real world.

Receiving a matching molecular-based therapy in our set of cases had a small impact on progression-free survival, and would be consistent with current grade I clinical evidence.[28] The absence of an observed PFS effect may be related to either patient selection, the effect of having a low ESCAT category as best therapeutic suggestion or, outside the context of oncogenic addiction, to suboptimal approaches that may just not consider most of the molecular landscape.

In our study, we did not observe that patients having a favourable ESCAT classification had a benefit in PFS. Very recently, the results of the SAFIR02-Breast clinical trial, which involved 238 patients with advanced breast cancer have been published. After receiving chemotherapy, patients were randomised to either maintenance targeted therapy matched to genomics or maintenance chemotherapy. In the subgroup of 115 patients with ESCAT I/II genomic alterations, PFS was statistically improved in patients receiving genomics-matched therapy. It should be remarked, however, that more than half of cases were related to BRCA gene or other homologous recombination deficiency alterations and received olaparib, and that generalising the results of this trial should be done with caution in other malignancies, as the authors suggest. Furthermore, when PFS was analysed in the overall population, no statistical differences were observed between treatment groups.[56] Another published trial of post-neoadjuvant maintenance therapy in 193 patients with residual triple negative primary breast cancer genomically-directed therapy was not superior to treatment of physician choice. The authors of this study suggest that NGS should not be used in the curative setting to guide therapy.[57] We found that the majority of our patients with an informed "druggable" gene alteration did not receive a matching therapy, and the most frequent situation was the inability to prescribe a drug outside of its approved indication. This has been reported by others as well,[46,58–60] and highlights the limitations of a "let's see what we find" approach. Therefore, we think that the routine use of NGS panel reports indicating targeted therapies outside of their indication should not be routinely encouraged. Regarding the number of present molecular druggable alterations (none versus one versus more than one), we did not find a relationship with PFS either (Supplementary Fig. S1). Previous reports have found a positive association between the presence of druggable alterations and clinical outcomes, but this may be the effect of the administration of one or more adequate matching therapies (including PARP or PD-1/L1 inhibitors for homologous-recombination deficient of high-tumour mutational burden),[56] or an over-representation of tumours with alterations associated with good clinical course regardless of a matching treatment, such as EGFR-mutant lung cancer.[42] In a real-world heterogenous mix of cases, with few druggable alterations-treatment matches, we did not find such trend. It is important to mention, however, that this conclusion has to be taken with caution because of the relatively small numbers of the subgroups.

NGS testing showed an association with improved outcomes in our series when it was performed for either making a therapeutic decision in initial advanced cancer -particularly lung cancer, clinical trial screening, or rare

cancers in early lines. Median PFS in these patients was almost a year (319 days). While the survival results in these patients may be due to patient selection (i.e., the type of performed analysis does not establish causality between performing the NGS test and improved PFS), and the observed outcomes may be related to performing the NGS testing in good PS cancer patient early in the course of metastatic assessment, they could provide a common-sense guideline for NGS testing.

Patients that were tested in more advanced disease, with poor PS, rapidly progressing tumours, or even in initial stages of advanced cancer when standard therapy was available (Fig. 4B and C) had a significantly poorer clinical course. We observed a poor prognosis in very advanced or rapidly progressing cancers, with a PFS time of only 116 days. This overlaps with the usual 16-week time to re-assess therapeutic efficacy in a patient in real-world clinics outside clinical trials –in other words, progression at the first evaluation. These observations should strengthen the validity of our initial recommendations, suggesting that performing an NGS test in such setting does not really change the clinical outcome compared to just administering standard of care or best supportive care.

The OS results shown in this study should be interpreted with great caution due to the relatively low numbers of individuals split in several different subgroups/categories, and patients, tumour types and targets heterogeneity. However, several reasons justify exploring the impact in OS, namely: 1) the timing of re-evaluation CT scans in real-world practice can vary and influence the measured PFS; 2) some patients with a reported druggable target may not receive a matching drug right away, but in subsequent lines; 3) patients with more than one druggable target may receive more than one matching alternative, and whereas one of them may not have a significant effect on each PFS interval, the serial combined administration of several matches may impact OS; and 4) some drugs and targets have a broader impact in OS than others (i.e., it is not the same to compare the effect of targeting mutant EGFR in lung cancer than prescribing everolimus for PIK3CA mutation in breast cancer, a common recommendation of the evaluated commercial panel). In order to avoid artifacts due to multiple comparisons, we restricted our analysis to the 3 comparisons shown in Fig. 5. Although the interpretation of OS analysis should be taken with caution, it seems to support the strength impact of clinical judgement categories in clinical outcomes (Fig. 5B and C).

Regardless of the observed differences in clinical outcomes, our study did not intend to conclude that there is a causality between ordering an NGS panel in a specific scenario and better or worse PFS/OS. Categories were not placed in the "useful" or "non-useful" blocks in light of their effect in PFS, but the other way around: clinical judgement drove our choice about whether to place one or another category, and then PFS/OS were compared between groups. PFS and OS comparisons were aimed to provide support (or lack of it) to the classification that we have issued. Should the results have been different, the conclusions of our study would have leant more to the line of "better definition and characterisation of patients has yet to be achieved; clinical judgement in ordering NGS panels does not seem to be associated with clinical outcomes", but our clinical judgement would have not changed. A comparative trial in which NGS panels are ordered following one or another set of guidelines could allow gathering conclusive (causal) evidence; however, we think that it is unrealistic to expect such a trial. In the meanwhile, real-life evidence is the best that can be provided to support clinicians' decisions.

The last point that deserves attention is the general landscape of genomic panel testing. We found that a majority of NGS tests were ordered outside our own recommended guideline indications,[40] and that the two additional scenarios in which oncologists ordered NGS tests were either not clearly indicated or had no survival impact (Fig. 4). In light of the survival data, we strongly recommend against ordering an NGS panel in patients with cancer with very advanced situations, and also in patients with advanced cancers that are out of standard options although they have a good performance status in which NGS testing is not useful. We also do not recommend NGS testing in either early cancers undergoing definitive therapy or advanced cancers with an available standard non-molecular therapy, in which NGS has no use. The latter cases showed a poorer PFS compared to categories 1–4. Practitioners' education is critical to get familiarised with real world data of use and efficacy of NGS in order to make adequate judgements, particularly in end-of-life situations.

In summary, according to our data, current NGS testing can be useful and may be recommended in patients with advanced cancer when molecularly-guided therapy is most applicable, including lung cancer, some rare cancers, and patient selection for clinical trials. Along this line, results from the MOSCATO-01 study suggest that the molecular screening of patients with metastatic rare cancers may increase the therapeutic options.[61] In these situations, NGS testing should be performed early after metastatic disease is established, and patients should have good PS. NGS testing in early cancer or in advanced cancer when conventional therapy is well established renders the test unnecessary. In the opposite side of the scale, performing NGS late in the disease course when the expected lifetime of poor PS patients is low or the disease is progressing very rapidly makes the test not useful, since the prognosis is not changed by the test results. NGS testing is similarly not useful in terms of prognosis in advanced cancer with no standard options, even if the general PS is preserved. Up until recently, it could be argued that

patients with very advanced disease, with either good or poor PS, might have a NGS retesting since multiple lines of therapy might induce selective pressure leading to major changes in the genomic landscape. However, a very recent manuscript demonstrates that this is not the case, since repeated serial NGS profiling to a cohort of 231 advanced patients with cancer treated with multiple lines found that 99% of the biomarkers were preserved, suggested a limited evolution of the actionable cancer genome.[62] Their data strengthen our recommendation towards performing NGS testing as soon as possible in the metastatic setting.

Our use of real-world data is a novel application providing evidence for new precision oncology therapies, as has been recently suggested.[61] Our study, although limited in size, offers an overview of which are the most common situations in which practitioners order NGS panels, and sheds further light about the utility of performing NGS testing in advanced patients with cancer. ESCAT categories will likely evolve in the future and more useful mutation-drug associations will be established. Our study shows that the results of a NGS panel in advanced cancer may provide some survival benefit when it is done early in the course of advanced disease, but is of little efficacy in later settings. Our observations strengthen the need for improving the understanding of genomic-drug associations and generating high-quality real-world evidence.

In conclusion, NGS testing seems to be useful in providing benefit in the clinical course of advanced cancer when it is performed in patients which routinely need multiple molecular markers, patients with advanced rare cancers, or patients that are screened for clinical trials. NGS testing is of little efficacy in patients with rapidly progressing cancer with short expected lifetime or in patients with cancer that have standard therapy available without need of genomic profiling.

Contributors

NRL, JH and LGC ordered the majority of the tests and managed the patients analysed in this study. RC, RM and MQF contributed with additional patients. RC, NRL, RM, JM and MQF collected patients' data and cross-verified the information contained in the original medical records. MQF, RC, MJB and SM analysed and interpreted the data. All authors confirm that they had full access to all the analysed data, contributed to writing the manuscript, approved the final version, and agreed to submit for publication.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon request.

Declaration of interests

JM received honoraria for educational events sponsored by Eli Lilly and MSD. NRL received honoraria for lectures and presentations sponsored by Janssen, Pfizer and MSD (paid to the institution) and for participation in Data Safety Monitoring/Advisory Boards from Astra Zeneca, Clovis, MSD and GSK. LGC received honoraria for lectures or presentations sponsored by MSD, Astra Zeneca and Daiichi Sankyo, and a travel grant from Eli Lilly. MQF has signed research contracts with Circle Therapeutics and MECO Diagnostics (funds paid to the institution). MQF has also received consulting fees from Exscientia and payments for educational events sponsored by Astra Zeneca, plus a travel grant from Pfizer. RC, RM, and MQF are faculty of the UAM-Fundación Instituto Roche endowed chair of Personalised Precision Medicine at the Universidad Autonoma de Madrid.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102029.

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