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Genomics to select treatment for patients with metastatic breast cancer

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Cancer progression is driven in part by genomic alterations[1]. The genomic characterization of cancers has shown interpatient heterogeneity regarding driver alterations[2], leading to the concept that generation of genomic profiling in patients with cancer could allow the selection of effective therapies[3,4]. Although DNA sequencing has been implemented in practice, it remains unclear how to use its results. A total of 1,462 patients with HER2-non-overexpressing metastatic breast cancer were enroled to receive genomic profiling in the SAFIR02-BREAST trial. Two hundred and thirty-eight of these patients were randomized in two trials (nos. NCT02299999 and NCT03386162) comparing the efficacy of maintenance treatment[5] with a targeted therapy matched to genomic alteration. Targeted therapies matched to genomics improves progression-free survival when genomic alterations are classified as level I/II according to the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT)[6] (adjusted hazards ratio (HR): 0.41, 90% confidence interval (CI): 0.27–0.61, $P < 0.001$), but not when alterations are unselected using ESCAT (adjusted HR: 0.77, 95% CI: 0.56–1.06, $P = 0.109$). No improvement in progression-free survival was observed in the targeted therapies arm (unadjusted HR: 1.15, 95% CI: 0.76–1.75) for patients presenting with ESCAT alteration beyond level I/II. Patients with germline *BRCA1/2* mutations ($n = 49$) derived high benefit from olaparib (g*BRCA1*: HR = 0.36, 90% CI: 0.14–0.89; g*BRCA2*: HR = 0.37, 90% CI: 0.17–0.78). This trial provides evidence that the treatment decision led by genomics should be driven by a framework of target actionability in patients with metastatic breast cancer.

Cancer is a disease whose development is at least partially driven by germline and/or somatic genetic alterations located on oncogenes or tumour suppressor genes[1]. Thanks to the advent of next-generation sequencing (NGS), DNA analyses have shown that the genomic drivers of cancer can differ between patients[2]. This observation led to the development of cancer precision medicine, in which a comprehensive genomic profile is generated in each patient and a targeted therapy is given accordingly[3,4]. Questions remain on how to use the results obtained from genomic profiling in daily practice, for treatment decision. The SAFIR02-BREAST trial is a prospective randomized trial that compared targeted therapies matched to genomic alterations with the standard of care—that is, maintenance chemotherapy[5]—in patients with metastatic breast cancer (mBC). Here we show that targeted therapies matched to genomics improves progression-free survival (PFS) when genomic alterations are classified as level I/II according to the European Society of Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT)[6] (adjusted HR: 0.41, 90% CI: 0.27–0.61, $P < 0.001$), but not when alterations are classified beyond level II (unadjusted HR: 1.15, 95% CI: 0.76–1.75). This trial provides evidence that the treatment decision led by genomics should be driven by a framework of target actionability in patients with mBC.

After the pilot study (SAFIR01) showed the feasibility of multi-gene technologies at a national level[7], the phase II trial (MOSCATO) suggested that, in 33% of patients, PFS was increased by therapy matched to a genomic alteration detected by a multi-gene panel[8]. Other trials reported consistent results, that around 20% of patients would gain access to new drugs and one-third of them would derive some benefit from such drug access[9]. Although these results show that sequencing

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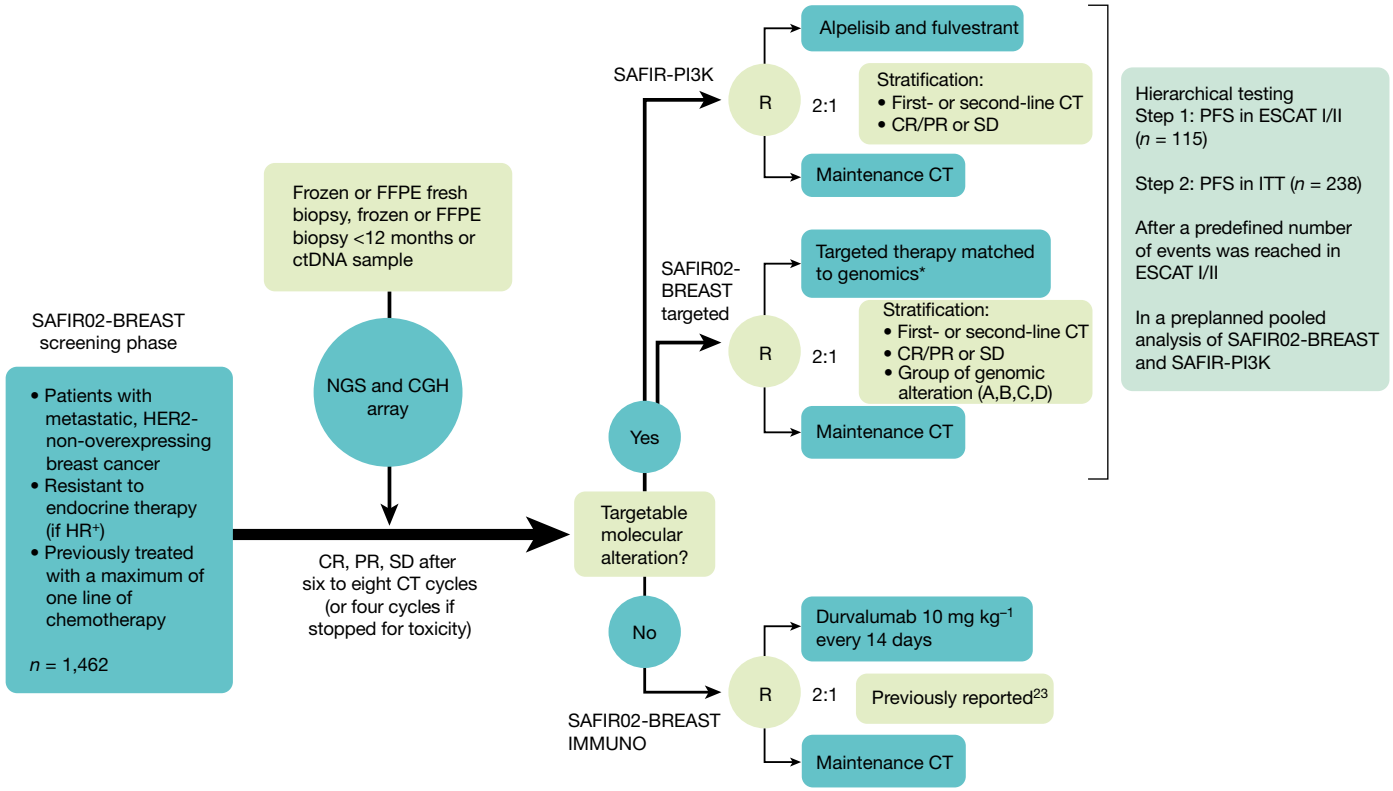

Fig. 1 | Design of the trial. Patients were selected on the basis of having received fewer than two lines of chemotherapy (CT) in the metastatic setting, and to present with mBC with no overexpression of HER2. After patients had signed an informed consent, a biopsy was done with the exception of (1) patients who had undergone a biopsy <12 months before inclusion and (2) patients for which a biopsy was not feasible. In the latter case, a plasma sample was obtained for analysis of circulating tumour DNA. Multi-gene sequencing and comparative genomic hybridization (CGH) arrays were performed as described in Methods. In patients eligible for germline sequencing, determination of germline *BRCA1/2* was also performed. Patients received between six and eight cycles of chemotherapy. For patients who presented with stable disease (SD) or an objective response (partial (PR) or complete (CR) response), and genomic alteration targetable by a drug available in the trial, randomization between matched targeted therapy and maintenance chemotherapy was proposed. The primary endpoint of the trial was PFS in a pooled analysis of SAFIR02-BREAST and SAFIR-PI3K. The trial had hierarchical testing, starting with the population of patients with ESCAT I/II alterations followed by the overall population.

technologies present good analytical validity and could improve outcome in a subset of patients, their impact and how to use the results in daily practice remain unclear. As an example, matching molecular alterations with drugs approved in another disease did not improve outcome in a randomized trial[10]. To prioritize genomic alterations for treatment decision, scales of evidence for genomic alterations have been developed. As an example, ESMO has created ESCAT[6], a scale that includes six levels ranked according to the level of evidence of the matched genomic alteration/drug. Level I means that the drug matched to the genomic alteration has been proved to be effective; level II means that the drug matched to the genomic alteration has been associated with preliminary evidence of efficacy. Beyond level II, the efficacy of the matched drug/genomic alteration is hypothetical at best. In the SAFIR02-BREAST trial in patients with mBC, we evaluated the clinical utility of the identification of therapeutic targets by genomics, together with the utility of the method in interpretation of the genomic report. The study design is shown in Fig. 1.

Results

Between April 2014 and October 2019, 1,462 patients with human epidermal growth factor receptor-2 (HER2)-negative mBC signed informed consent to perform a biopsy and/or a genomic test in SAFIR02-BREAST, and were included in the trial. Out of 1,462 screened patients, 646 presented with a targetable genomic alteration. The CONSORT diagram and genomic alterations are reported in Extended Data Figs. 1 and 2, respectively. Two hundred and thirty-eight (16%) patients were subsequently randomized between maintenance chemotherapy ($n = 81$) and targeted therapy ($n = 157$). The clinical characteristics of the 238 patients randomized, together with the drugs used in the maintenance setting, are reported in Table 1.

The first step in the statistical plan was to analyse the efficacy of targeted therapies in patients presenting with an ESCAT I/II genomic alteration. Of the 115 patients with a genomic alteration classified as ESCAT I/II, 57 presented with *BRCA1/2* alterations, three with a *PALB2* alteration, 31 with a *PIK3CA* mutation, 16 with an *AKT1* mutation, five with a *PTEN* mutation and/or deletion and three with an *ERBB2* mutation. Eight patients presented with a somatic-only *BRCA1/2* genomic alteration. *BRCA* genomic alteration was determined by germline sequencing in 13 patients (including six for which NGS/comparative genomic hybridization (CGH) was not feasible). Adverse events were pooled for ESCAT I/II, and intent-to-treat (ITT) populations and are reported in Supplementary Data 1. No new safety signal was detected. After a median follow-up of 21.4 months (90% CI: 17.9–27.6), analysis of the primary endpoint in patients with ESCAT I/II showed a significantly longer PFS in the targeted therapy arm than in the control arm. Median PFS was 9.1 months (90% CI: 7.1–9.8) in the experimental arm and 2.8 months (90% CI: 2.1–4.8) in the control arm (adjusted HR = 0.41, 90% CI: 0.27–0.61, $P < 0.001$) (Fig. 2a). We further assessed the efficacy of matching an ESCAT I/II genomic alteration with targeted therapy within subgroups (Extended Data Fig. 3). The efficacy of matched targeted therapies was not

Table 1 | Patient characteristics

	Overall population (*n*=238)			ESCAT I/II population (*n*=115)		
	Arm A (*n*=157)	**Arm B (*n*=81)**	***P* value**	**Arm A (*n*=75)**	**Arm B (*n*=40)**	***P* value**
Breast cancer subtypes of primary tumour						
TNBC	32 (21%)	19 (25%)	0.54	19 (28%)	13 (35%)	0.68
$HR^+/HER2^-$	116 (77%)	56 (75%)		50 (71%)	24 (65%)	
$HER2^+$	3 (2%)	0		1 (1%)	0	
Missing	6	6		5	3	
Previous chemotherapy in metastatic setting						
Yes	93 (59%)	45 (56%)	0.58	50 (67%)	24 (60%)	0.48
No	64 (41%)	36 (44%)		25 (33%)	16 (40%)	
Previous hormonotherapy in metastatic setting						
Yes	73 (46.5%)	34 (42%)	0.50	37 (49%)	16 (40%)	0.34
No	84 (53.5%)	47 (58%)		38 (51%)	24 (60%)	
Number of cycles of chemotherapy received at randomization						
Four or five	10 (6%)	5 (6.5%)	1.00	5 (7%)	3 (8%)	0.52
Six to eight	146 (93%)	75 (92.5%)		70 (93%)	36 (90%)	
Nine	1 (1%)	1 (1%)		0	1 (2%)	
Number of metastatic sites at screening						
Under three	77 (49%)	43 (53%)	0.55	36 (48%)	20 (50%)	0.84
Three or more	80 (51%)	38 (47%)		39 (52%)	20 (50%)	
Liver metastasis at screening						
Yes	101 (64%)	53 (65%)	0.87	46 (61%)	23 (57.5%)	0.69
No	56 (36%)	28 (35%)		29 (39%)	17 (42.5%)	
Line of chemotherapy at randomization						
First	132 (84%)	69 (85%)	0.82	65 (87%)	38 (95%)	0.21
Second	25 (16%)	12 (15%)		10 (13%)	2 (5%)	
Disease status at randomization						
Partial/complete response	74 (47%)	38 (47%)	0.97	34 (45%)	18 (45%)	0.97
Stable disease	83 (53%)	43 (53%)		41 (55%)	22 (55%)	
Matched genomic alteration/targeted therapy decision by MTB						
IGF1R amplification (*n*=3), TSC1/2 mutation (*n*=3), STK11 deletion (*n*=1), RPTOR amplification (*n*=1) and AZD2014	7 (4%)	1 (1%)	–	NA	NA	–
AKT1 mutation and AZD5363	12 (8%)	4 (5%)		12 (16%)	4 (10%)	
PTEN mutation and/or deletion and AZD5363	8 (5%)	2 (2%)		4 (5%)	1 (3%)	
PIK3CA (*n*=36), PIK3R1 (*n*=2); mutations/PIK3CB (*n*=1), AKT1 (*n*=1), AKT3 (*n*=1), PDPK1 (*n*=1); amplifications and AZD5363	30 (19%)	12 (15%)		NA	NA	
FGFR1 (*n*=19), FGFR2 (*n*=1), FGF4 (*n*=4), FGFR3 (*n*=1); amplifications/ FGFR2 (*n*=1), FGFR4 (*n*=1); mutations and AZD4547	17 (11%)	10 (12%)		NA	NA	
ERBB2 mutation and AZD8931	1 (0.5%)	2 (2%)		1 (1%)	2 (5%)	
EGFR mutation/amplification (*n*=3) or ERBB3 mutation (*n*=2) and AZD8931	2 (1%)	3 (4%)		NA	NA	
FRS2 amplification (*n*=10), NF1 mutation (*n*=7), KRAS mutation (*n*=4), BRAF amplification (*n*=1), BRAF mutation (*n*=1) and selumetinib	17 (11%)	6 (7%)		NA	NA	
VEGFA amplification (*n*=3), RET mutation (*n*=1), EGFR amplification (*n*=1), KDR mutation (*n*=1) and vandetanib	3 (2%)	3 (4%)		NA	NA	
AR amplification and bicalutamide	0	1 (1%)		NA	NA	
BRCA1 mutation and olaparib	11 (7%)	10 (12%)		11 (15%)	10 (25%)	
BRCA2 mutation and olaparib	26 (16%)	10 (12%)		26 (35%)	10 (25%)	
PALB2 mutation and olaparib	1 (0.5%)	2 (2%)		1 (1%)	2 (5%)	
ATR/ATM mutation/deletion and olaparib	2 (1%)	4 (5%)		NA	NA	
PIK3CA mutation and alpelisib	20 (13%)	11 (14%)		20 (27%)	11 (27%)	
ESCAT ranking						

Continued

	Overall population (*n*=238)			ESCAT I/II population (*n*=115)		
	Arm A (*n*=157)	Arm B (*n*=81)	*P* value	Arm A (*n*=75)	Arm B (*n*=40)	*P* value
I	49 (31%)	31 (38%)	0.22	49 (65%)	31 (77%)	0.18
II	26 (17%)	9 (11%)		26 (35%)	9 (22%)	
III	15 (10%)	4 (5%)		NA	NA	
IV	27 (17%)	18 (22%)		NA	NA	
X	10 (6%)	9 (11%)		NA	NA	
NM	30 (19%)	10 (12%)		NA	NA	
Chemotherapy administered in the maintenance setting (*n*=63)						
Paclitaxel	NA	21 (33%)	NA	NA	12 (36%)	NA
Capecitabine	NA	13 (21%)		NA	5 (15%)	
Bevacizumab	NA	8 (13%)		NA	6 (18%)	
Paclitaxel + bevacizumab	NA	7 (11%)		NA	3 (9%)	
Other	NA	14 (22%)		NA	7 (21%)	

NM, not matched; NA, not applicable.

different between patients with ESCAT I (HR = 0.53, 90% CI: 0.34–0.82) and ESCAT II genomic alterations (HR = 0.38, 90% CI: 0.18–0.78). Patients with germline *BRCA1/2* mutations derived high benefit from olaparib (g*BRCA1*: HR = 0.36, 90% CI: 0.14–0.89; g*BRCA2*: HR = 0.37, 90% CI: 0.17–0.78). There was no heterogeneity between genomic alterations regarding treatment efficacy (interaction test, *P* = 0.26) (Extended Data Fig. 3). When patients with g*BRCA1/2* alterations were excluded from the analysis (*n* = 66), HR for progression or death was 0.64 (90% CI: 0.39, 1.06). Although median PFS was 1.8 (90% CI: 1.4–NR (not reached)) and 2.3 months (90% CI: 1.8–2.8) in the control arms of cohorts AZD5363 and olaparib, it was 8 months (90% CI: 3.2–15.5) in the cohort alpelisib. This could reflect the fact that a large number of patients received effective chemotherapy in the maintenance setting in this cohort (paclitaxel, *n* = 4, 44%; capecitabine, *n* = 4, 44%; cyclophosphamid and epirubicin, *n* = 1, 11%). Targeted therapies matched to ESCAT I/II genomic alterations had no impact on overall survival (adjusted HR = 0.94, 90%CI: 0.57–1.55, *P* = 0.831) when given in the maintenance setting.

Because genomics had an impact on PFS in the subgroup of patients with ESCAT I/II, we moved the statistical analysis to the second step. In the overall population, with median follow-up of 24.7 months (95% CI: 17.9–30.6), there was no significant difference in PFS between the two arms (adjusted HR: 0.77, 95% CI: 0.56–1.06, *P* = 0.109). Median PFS in the control arm was 2.9 (95% CI: 2.3–4.8) and 5.5 (95% CI: 4.0–6.9) months in the targeted therapy arm (Fig. 2b). ESCAT classification was highly predictive for the benefit of targeted therapies matched to genomic alterations (interaction test, *P* = 0.004; Extended Data Fig. 4). Targeted therapies matched to genomic alterations were not effective in patients without ESCAT I/II alteration (HR: 1.15, 95% CI: 0.76–1.75). In this subgroup, median PFS was 2.8 (95% CI: 1.6–4.2) and 3.1 (95% CI: 1.8–5.7) in patients treated with targeted therapy and maintenance chemotherapy, respectively (*P* = 0.49) (Fig. 2c). We could not detect any evidence that a genomic alteration ranked III/IV was associated with outlier sensitivity to a matched therapy. Indeed, only one patient with an ESCAT III/IV alteration and treated with targeted therapy had PFS > 12 months.

Taking advantage of the large number of patients included in the trial, we then performed exploratory biomarker analyses. In multivariable analyses that included age and grade, number and site of metastases, *TP53* mutations (adjusted HR: 1.87, 95% CI: 1.49–2.34, *P* < 0.001), homologous recombination deficiency (HRD) (adjusted HR: 1.31, 95% CI: 1.05–1.63, *P* = 0.017) and *PIK3CA* mutations (adjusted HR: 1.25, 95% CI: 1.00–1.56, *P* = 0.052) were associated with poor overall survival from inclusion in the population of patients presenting with hormone receptor (HR)$^+$/HER2$^-$ mBC (*n* = 574 for CGH and 614 for NGS; Supplementary Data 2).

We then focused the analyses on patients who presented with a *BRCA1/2* alteration, treated with olaparib and for which a copy number analysis was done on the tumour sample (*n* = 31). In this group of patients, loss of heterozygosity (LOH) on *BRCA1* and/or *BRCA2* was associated with longer PFS (HR = 0.32, 95% CI: 0.14, 0.73, *P* = 0.0049) (Fig. 3a). LOH on *BRCA1/2* was associated with higher HRD (*P* < 0.001). A high HRD score (≥42) was associated with a better outcome (HR = 0.32, 95% CI: 0.12, 0.83, *P* = 0.013) (Fig. 3b). Two patients presenting with an *AKT1* mutation and treated with capivasertib had PFS censored at 24 and 27 months, respectively. Two patients with *PTEN* alteration and treated with capivasertib had PFS of 12 and 23 months, respectively; one of these patients had Cowden syndrome and was previously reported[11]. The second patient presented with two somatic *PTEN* mutations associated with a hotspot loss-of-function mutation on *PIK3R1*, a gene coding the regulatory subunit of PI3K[12]. Patients presenting with a double *PIK3CA* mutation (*n* = 5) and treated with alpelisib and fulvestrant had a lower hazard of progression (HR = 0.34, 95% CI: 0.07, 1.66) compared with patients having a single mutation (*n* = 10). One patient with a double *PIK3CA* mutation and treated with alpelisib and fulvestrant was censored at 30 months. Finally, we assessed which genes were more frequently amplified or deleted in mBC compared with primary tumours. We first analysed the 45 genes previously reported by Curtis[13] to present recurrent copy number alterations in early-stage breast cancers. In patients with HR$^+$/HER2$^-$ mBC (*n* = 565), 14 of these genes presented copy number alterations more frequently in mBC as compared with early-stage breast cancer (*n* = 2,162) (Fig. 3c). When we analysed the entire genome, five additional genes were more frequently amplified in HR$^+$/HER2$^-$ mBC as compared with early-stage breast cancers (Fig. 3c). TERT (telomerase reverse transcriptase) was amplified in 5.8% of HR$^+$/HER2$^-$ mBC. No gene was significantly more frequently amplified or deleted in metastatic triple-negative breast cancer (TNBC) (*n* = 361) as compared with early-stage TNBC (*n* = 471).

Discussion

Although progress is providing newer sequencing tools that potentially overcome technological hurdles, our study shows that the tools for clinical interpretation of sequencing are pivotal in achieving tangible clinical benefits. In the current study we tested ESCAT as a framework for actionability[6,10] and found that DNA sequencing should lead to treatment administration only for patients presenting with genomic alterations ranked level I/II. This could extrapolate to the tier I and II evidence from the Association for Molecular Pathology[14] and to levels 1–3A from OncoKB[15]. The finding that such frameworks constitute the pillar for an effective implementation of cancer precision medicine is an argument for investment in the development of databases that inform clinical teams about the relevance of matched drug–genomic alterations[15,16].

Pioneer publications on cancer precision medicine have reported the vision that modelling biology in each patient, on the basis of DNA

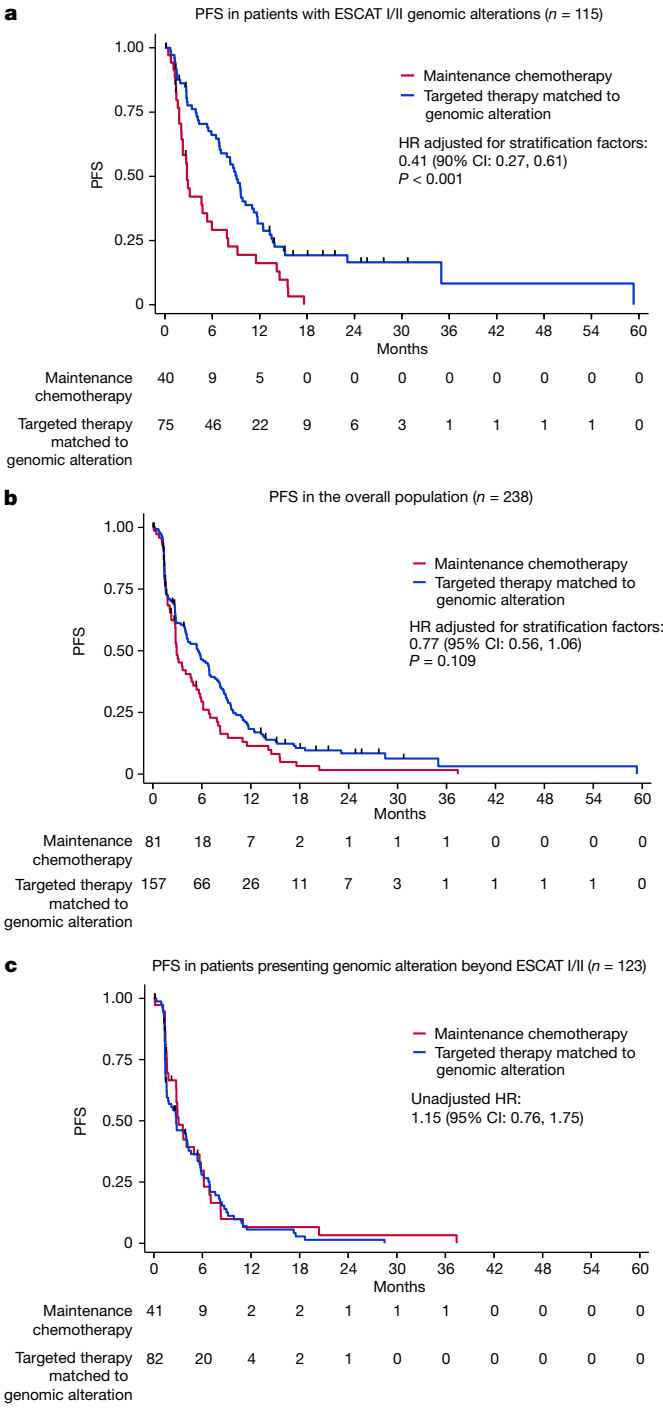

Fig. 2 | PFS according to treatment arm and ESCAT ranking. a–**c**, PFS in patients with ESCAT I/II (**a**), in the overall population (**b**) and with alteration beyond ESCAT I/II (**c**), according to treatment arm. HR and *P* values were estimated using a Cox proportional-hazards model adjusted to stratification factors in patients with ESCAT I/II (**a**) and in the overall population (**b**) (primary objectives), and are unadjusted in patients with alteration beyond ESCAT I/II (**c**). HR is reported, together with 90% CI for the ESCAT I/II population and 95% for the overall and non-ESCAT I/II populations. All statistical tests were two-sided. No adjustment was made for multiple comparisons.

sequencing, would allow identification of therapeutic targets at the individual level[4,17]. Our study suggests that, besides the companion diagnostics validated in therapeutic trials, we do not yet have the biological knowledge to identify a target in each individual. Indeed, in the SAFIR02-BREAST trial there was no efficacy of targeted therapies when genomic alterations were classified beyond level II. This finding is

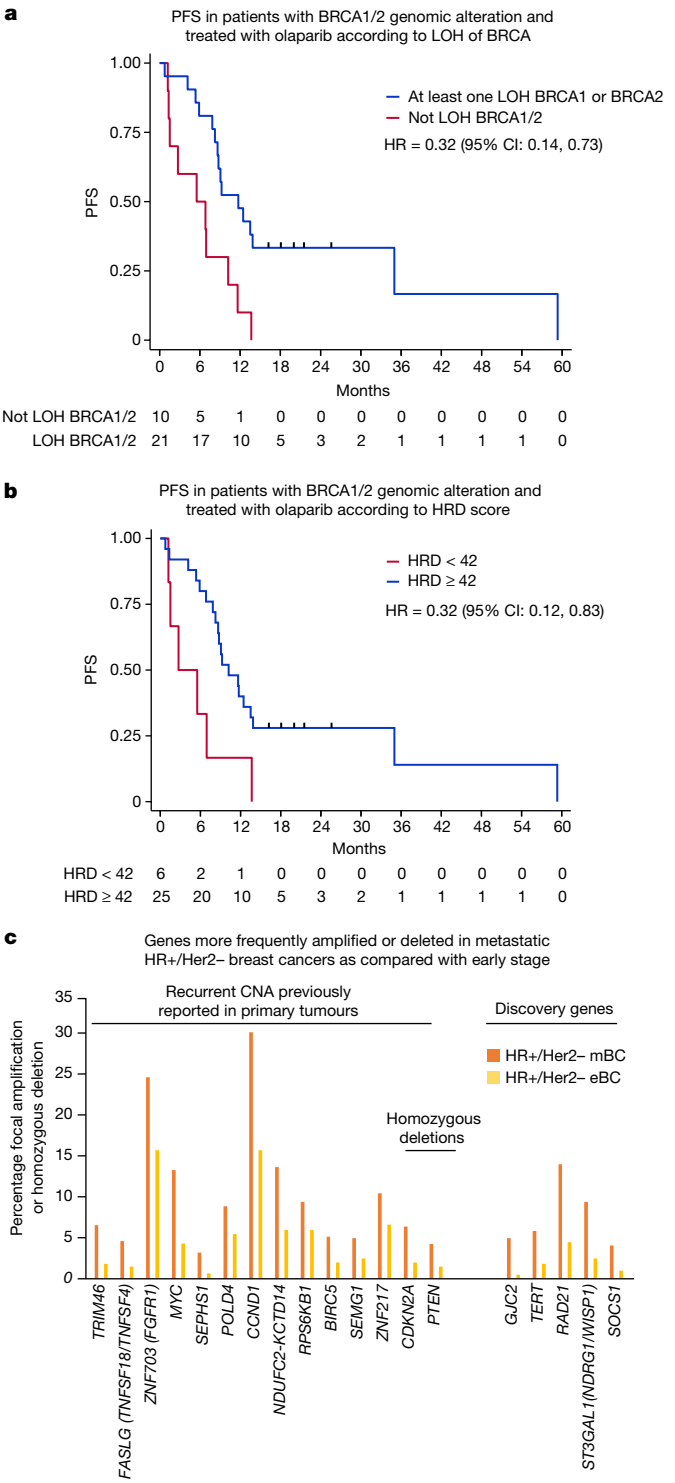

Fig. 3 | Exploratory analyses on biomarkers. a, PFS according to the presence of LOH on BRCA, as defined in Methods. **b**, PFS according to the presence of HRD: a cut-off was predefined at 42, as reported in Methods. PFS was assessed in patients with *BRCA1/2* genomic alteration, treated with olaparib and for which a copy number analysis had been done on tissue. HR from the Cox proportional-hazards model is reported, together with 95% CI. **c**, The 19 genes found to present more frequently with a focal amplification or deletion in $HR^+/HER2^-$ mBC ($n = 565$) as compared with early-stage $HR^+/HER2^-$ breast cancers (early breast cancer (eBC)) ($n = 2{,}162$). The frequencies of copy number alterations reported in the figure are calculated from SAFIR02-BREAST (mBC) and TCGA (eBC). Genes were considered significant at $P < 0.05$ in comparison between SAFIR02-BREAST and all three early-stage BC datasets (TCGA, METABRIC and PACS04).

corroborated by preclinical data suggesting that DNA sequencing alone is not a tool suitable for the development of predictors of drug sensitivity[18]. The future in the field of cancer modelling will be to integrate, in each patient, data derived from different sources. As example, in the DREAM project, integration of RNA expression with DNA sequencing improved prediction for drug sensitivity[18]. Other research efforts are currently evaluating the use of organoids to model cancer biology and predict drug sensitivity[19,20].

Interestingly, the current trial suggests a high efficacy of olaparib given in the maintenance setting (HR = 0.36, 90% CI: 0.14–0.89) and HR = 0.37 (90% CI: 0.17–0.78) in patients with g*BRCA1* and g*BRCA2*, respectively), whereas its benefit was more limited in the OlympiaD trial in which it was administered as frontline therapy (HR = 0.58, 95% CI: 0.43–0.80)[21]. This finding suggests that there is a rationale to testing poly-(ADP-ribose) polymerase (PARP) inhibitors as maintenance therapy.

The current trial presents several limitations. First, it did not test a specific platform or technology of sequencing but rather the utility of multiple genomic tests and the method used to interpret these. This is common and is a contingency in clinical trials in which, over the time required to complete enrolment, newer or constantly refined sequencing platforms are implemented. Second, patients with germline *BRCA* mutations derive high benefit from matched targeted therapy and one could argue that the results from the ESCAT I/II group were driven by this population. Nevertheless, subgroup analysis shows that targeted therapies matched to genomic alterations also reduce the risk of progression when excluding patients with germline *BRCA1/2* mutations (HR = 0.64, 90% CI: 0.39, 1.06). Third, the design allowed the inclusion of only those patients who presented with sensitivity to chemotherapy. This could have biased the population toward a group of patients who do not present with genomic alterations involved in resistance. Fourth, some drugs could be considered suboptimal. As an example, neratinib, for which efficacy has been extensively reported[22], would have been a better choice to target HER2 as compared with AZD8931. Finally, the design itself did not properly test precision medicine because the control arm included maintenance chemotherapy.

The SAFIR02-BREAST trial suggests that the use of genomics improves the outcome of patients who present with a match drug/alteration ESCAT I/II, but not for those presenting with ESCAT beyond I/II. Reporting the results of genomics in the context of a framework of target actionability should therefore be considered as a standard of care. The results of the trial should be interpreted with caution, because a large part of the benefit observed with matched targeted therapies was derived from patients presenting with *BRCA1/2* alteration and the small sample size does not allow exploration of the actionability of new genomic alterations.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-022-05068-3.

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Methods

Patients and study design

SAFIR02-BREAST (no. NCT02299999), a prospective trial testing the clinical utility of genomics, was run between 2014 and 2021 (Supplementary Data 3 and 4). The study design is reported in Fig. 1. Patients were eligible if they presented with $HER2^-$ mBC and had received a maximum of one line of chemotherapy in the metastatic setting. Patients presenting with HR^+ breast cancer had to be resistant to endocrine therapy (tamoxifen or aromatase inhibitor). Patients who were eligible signed an informed consent for biopsy and genomic analyses within SAFIR02-BREAST. Patients were given a genomic test on either a biopsy of metastasis (n = 1,163) or a plasma sample obtained before the third cycle of chemotherapy (n = 125 between 2017 and 2019). Patients received conventional chemotherapy on the basis of the decision of the investigator. When genomic results were available, a national molecular tumour board (MTB) allocated the patient to a targeted therapy available in the trial (listed in Table 1). After six to eight cycles of chemotherapy, patients who did not present with progressive disease were randomized between the targeted therapy matched to genomic alteration as decided by the MTB, or maintenance of chemotherapy for a duration decided by the investigator. From December 2017, a specific protocol (SAFIR-PI3K, no. NCT03386162; Supplementary Data 5)) was opened for patients with $HR^+/HER2^-$ mBC presenting with a hotspot *PIK3CA* mutation detected in SAFIR02-BREAST. This protocol randomized alpelisib (300 mg) combined with fulvestrant (500 mg monthly) versus chemotherapy, and data were preplanned to be merged with SAFIR02-BREAST. All patients who entered the randomized part of SAFIR02-BREAST and SAFIR-PI3K signed informed consent. The SAFIR02-BREAST trial was approved by the French ethics committee, CPP, on 13 December 2013 (no. 2013-09-07) and the French health authorities, ANSM, on 14 January 2014 (no. 2013-001652-36). The SAFIR-PI3K trial was approved by CPP on 7 July 2017 (no. 2-17-21) and by ANSM on 19 July 2017 (no. 2017-000154-19). SAFIR-PI3K was introduced in the SAFIR02-BREAST trial in 2017 and was approved by CPP on 17 September 2017 (no. 2013-09-07 MS10) and by ANSM on 22 December 2017 (no. 2013-001652-36 MS10). An Independent Data Monitoring Committee (IDMC) that included six members monitored the study every 6 months.

Molecular testing and reporting

ER (estrogen receptor), PR (progesteron receptor) and HER2 (human epidermal growth factor receptor 2) were determined locally. The last status available was used to define molecular subtypes.

A tumour sample was obtained either prospectively after signing the informed consent or retrospectively if the last biopsy was performed <12 months before inclusion. When it was not possible to perform biopsy or when tumour samples could not be analysed (<30% tumour cells for frozen sample, <10% for formalin-fixed, paraffin-embedded (FFPE) samples), circulating tumour DNA was used to perform genomic profiling. Methods for DNA extraction are previously reported[23]. Five core facilities performed the genomic analyses. CytoScan and OncoScan FFPE Assays Kits (Affymetrix, Thermo Fisher Scientific) were used to determine copy number alterations in fresh tumour DNA and FFPE or ctDNA samples, respectively. Two gene panels were used during the trial: the first assessed 50 genes and was used between 2014 and 2017; the second assessed 65 genes and was used after 2017 (list of genes is given in Supplementary Material and Study Protocol, Supplementary Data 4). DNA (10 ng) was used to perform the initial PCR step (17 cycles). Amplicons were then partially digested using the enzyme FuPA to eliminate extremities corresponding to primer sequences. The digested product was ligated with adaptors and barcodes, then amplified and purified. Adaptors contained specific indices (barcodes) different for each sample so that libraries from different samples could be combined before sequencing. Quality and quantity assessment of DNA libraries was achieved using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and/or BioAnalyzer profiling. After equimolar pooling of libraries, the final solution was sequencing using either a MiSeq instrument (Illumina), an Ion Torrent PGM or an Ion Torrent GeneStudio S5 (Thermo Fisher Scientific), depending on the various regional molecular cancer genetics platforms. A depth of coverage of >100 reads was required for variant calling, with thresholds of 5% for the alternate allele for calling of SNVs/mutations and 10% for indels. Raw reads were aligned on the reference human genome hg19, and variants were annotated using ANNOVAR and the following databases: COSMIC68, dbSNP137, 1000 Genomes, ESP6500 and RefGene annotations. Only non-synonymous variants not observed in >0.1% of the population (1000 Genomes, ESP6500 and gnomAD) were identified as mutations. All somatic mutations were annotated, sorted and interpreted (pathogenic, probably pathogenic or unlikely to be pathogenic) by an expert in molecular biology according to available databases (Cosmic, TCGA, cancer Hotspot, OncoKB). Only truncating mutations (frameshift deletion and insertion, stop gain, splicing alteration) and known mutations (missense variants and in-frame deletion and insertion) from available databases (Cosmic, TCGA, cancer Hotspot, OncoK) were retained for tumour suppressor gene pathogenic variants. All missense variants and in-frame deletions and insertions known to be hotspot mutations from available databases (Cosmic, TCGA, cancer Hotspot, Onco) were retained for oncogene pathogenic variants.

Copy number variations (CNVs) from CytoScan and OncoScan Affymetrix were defined using the R package rCGH[24]. Briefly, $\log_2$ relative ratios (LRRs) were calculated and centralization of the profile determined the baseline (two copies being the neutral level) from which copy number analysis (CNA) was estimated. Breakpoints in LRR continuity were identified by segmenting the profile. These segments were further used to determine potential gains or losses, using the following scale: copy = 0, homozygous deletion; copy = 1, loss; copy = 2, copy neutral; 3 ≤ copy ≤4, gain; copy > 4, amplification. The type of LOH state was defined using the R package EaCoN, available at github (https://github.com/gustaveroussy/EaCoN). HRD score[25] was determined using HRDetect. Based on the CNV profile, HRDetect measures the frequency of large-scale LOH, telomeric allelic imbalance and large-scale transition events to determine HRD score. A cut-off of 42 was selected for HRDetect before analyses[26]. To perform comparison of CNAs between mBC and early-stage BC (TCGA[2], METABRIC[13] and PACS04, a prospective trial testing adjuvant chemotherapy[27]), only focal amplifications and homozygous deletions were considered. For both early-stage BC and mBC samples, we excluded those that (1) were ctDNA; (2) failed during processing; (3) had a flat (low-dynamic) CGH profile; (4) were considered too noisy (a threshold of 1,500 segments by profile was used to considered a sample as noisy); or (5) showed *ERBB2* amplifications. Based on these parameters, we compared 926 mBC versus 2,633 early-stage BC. CNA was performed in the same way for early-stage BC as for mBC. Focally amplified genes were defined as those fully included in a DNA segment <10 Mb and with copy number six or greater. Homozygous deleted genes were defined as those fully or partially included in a DNA segment with a copy number of 0. For each focal amplified region we identified gene(s) located in the smallest common focal amplified DNA segment (SCFADS). If several genes located were in this SCFADS, we have indicated those from the OncoKB Cancer Gene List (https://www.oncokb.org/cancerGenes). The frequency of amplification and/or deletion in SAFIR02-BREAST was compared with that of the three early-stage BC datasets. Genes were considered significant at $P < 0.05$ in the comparison between SAFIR02-BREAST and all three early-stage BC datasets (TCGA, METABRIC, PACS04). For TNBC, only TCGA and METABRIC were used for comparison.

The genomic results of each patient were discussed during a bimonthly MTB, when allocation to a targeted therapy arm was decided.

Treatment and follow-up

The experimental arm included eight drugs in SAFIR02-BREAST (capivasertib (AZD5363) 960 mg, 4 days on/3 days off; vistusertib (AZD2014) 100 mg, continuous dosing; AZD8931 80 mg, continuous dosing; AZD4547 160 mg, 2 weeks on/1 week off; olaparib 600 mg, continuous dosing; selumetinib 150 mg, continuous dosing; bicalutamide 150 mg, continuous dosing; vandetanib 300 mg, continuous dosing); and one combination (alpelisib 300 mg d^{-1} combined with fulvestrant 500 mg monthly) in SAFIR-PI3K. Recommendations for dose reduction are reported in V1 of the SAFIR02-BREAST protocol (Supplementary Data 3) and in the SAFIR-PI3K protocol (Supplementary Data 5). Treatments were given until either progressive disease or unacceptable toxicity, as defined by the investigator. In the control arm, the investigator continued the chemotherapy given during the first six to eight cycles. The plan was to continue chemotherapy until either disease progression or unacceptable toxicity. The decision to stop chemotherapy was given at the discretion of the investigator. The patient was censored if a switch was performed from investigational drug or chemotherapy to another drug in the maintenance setting (with the exception of tamoxifen or aromatase inhibitors in patients with endocrine-resistance HR^+ mBC). Matched drug–genomic alterations, together with ESCAT ranking and numbers of patients, are reported in Table 1. An assessment of drug efficacy was done every 6 weeks for the first 6 months, and thereafter every 9 weeks. Response was defined according to RECIST1.1 criteria. Based on recommendations from the IDMC, clinical progressions were also included as events for primary endpoint.

Statistical analyses

The primary objective of the SAFIR02-BREAST trial was to show that targeted therapies guided by genomic analysis improve PFS as compared with standard maintenance using cytotoxic therapy. The primary endpoint of the SAFIR02-BREAST trial is PFS in a pooled analysis of SAFIR02-BREAST and SAFIR-PI3K protocols. The initial protocol (Supplementary Data 3) was aimed at comparison of genomic-driven targeted therapies with maintenance chemotherapy in the ITT population. This protocol was amended in October 2020 following the release of the ESCAT ranking system, to perform hierarchical testing as described below (approved by CPP on 23 December 2020 (no. 2013-09-07 MS17), ANSM on 13 November 2020 (no. 2013-001652-36 MS17) and the IDMC). ESCAT I/II alterations were those reported in 2019 by Condorelli et al.[28], updated with Tung et al.[29] for PALB2 and somatic BRCA1/2 alterations (ESCAT II)[29].

Comparisons of PFS between arms were planned to be tested with a hierarchical fixed-sequence procedure in prespecified populations: step 1 in the ESCAT I/II population with a two-sided level of 10% significance, followed by step 2 in the ITT population at a two-sided level of 5% significance. Statistical significance was required at step 1 before formal testing of step 2, otherwise comparison in the ITT population was considered exploratory. Full details about the hierarchical procedure are provided in Supplementary Data (protocol and statistical analysis plan, Supplementary Data 4).

For the primary objective of PFS in the ESCAT I/II population, 85 events of tumour progression or death would be needed in this subgroup of patients (with 90% power, a two-sided significance level of 10% and a 2:1 randomization ratio) to detect a HR of 0.51 (increase in median PFS from 3.00 to 5.88 months). For the ITT population, 205 events of tumour progression or death would be needed (with 80% power, a two-sided significance level of 5% and a 2:1 randomization ratio) to detect a HR of 0.66 (increase in median PFS from 3.00 to 4.54 months). To observe the number of events required, we estimated that the trial needed to randomize 240 patients with at least 110 in ESCAT I/II categories. Based on PFS observed during the chemotherapy phase and the number of genomic abnormalities identified in SAFIR01 (ref. [6]), we planned to screen 1,460 patients to achieve these objectives.

Primary endpoints were planned for analysis on the ESCAT I/II and ITT populations (targeted substudy 1) when the required number of events had been reached (n = 85 for ESCAT I/II and n = 205 for overall population). In agreement with the IDMC, it was decided to analyse the primary endpoint of the study after 85 events in ESCAT I/II and after 195 events in the ITT population. Treatment allocation was performed using the minimization method as implemented in the randomization module of eCRF (ennov Clinical).

The primary endpoint (PFS) and secondary endpoint (overall survival (OS)) were analysed with a Cox regression model, adjusted for the variables used for stratification of randomization (line of chemotherapy, disease status at randomization and group of genomic alteration). The magnitude of the treatment effect was estimated with the adjusted HR and its 90% CI on the ESCAT I/II population and 95% CI on the ITT population. The Kaplan–Meier approach was used to estimate survival rates for each treatment arm. For subgroup analyses, treatment differences were tested using the log-rank test and HR estimated with an unadjusted Cox proportional-hazards model. Statistical significance of the interaction between treatment effect and a covariate was tested using a Cox proportional-hazards model fitted with the covariate, the treatment arm and an interaction term between the treatment arm and covariate. For exploratory biomarker analyses regarding *TP53*, HRD and *PIK3CA* mutations, OS was defined as the time from inclusion to death and was estimated using the Kaplan–Meier method. Univariable and multivariable analyses were performed using the log-rank test and Cox proportional-hazards model, respectively. Factors with $P \leq 0.2$ in univariable analysis were included in multivariable analysis. Statistical analyses were carried out using Stata software v.16 (StataCorp).

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Genomic data and modalities for access are available at EGAS00001005584 and https://nextcloud.gustaveroussy.fr/s/JXLt-7taZs8EtBF7.

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Author contributions F.A., T.B., T.F. and M.J. designed the study. F.A. and T.B. were the coordinators of the SAFIR02-BREAST trial. F.A. and A.G. were the coordinators of the

SAFIR-PI3K trial. M.J. and A.J. operated the trial. T.B., M.A., F.D., M.-P.S., M.C., H.B., C.L.-P., W.J., F.C., J.-M.F., C.L.-P., M.-A.M.R., J.-C.T., N.I., A. Mege, P.B., B.Y., N.H. and A.G. included patients in the trial. I.B., L.L., E.R., S.B., V.A., I.S., M.K., C.L., N.S., P.G., L.L.C. and A. Morel performed genomic analyses. A.T.-D. collected genomic data and stored them on EGA. F.A., T.B., F. Mosele, T.F., A.L., I.B., M.J., F. Montemurro and A.J. wrote the manuscript. All authors have read and agreed on the content of the manuscript.

Competing interests F.A. received research funding and served as a speaker/advisor (compensated by the hospital) for Pfizer, Roche, Lilly, Daiichi Sankyo, AstraZeneca and Novartis. T.B. received research funding and served as a speaker/advisor (compensated by the hospital) for Roche, Novartis, Pfizer, Seattle Genetics, Lilly and AstraZeneca. M.A. received research funding and served as a speaker/advisor (compensated by the hospital) for Novartis, AstraZeneca, Seattle Genetics, Abvie and Pfizer. M.C. received research funding and served as a speaker/advisor (compensated by the hospital) for AstraZeneca, Novartis, Abbvie, Sanofi, Lilly, Pfizer, Sandoz, ACCORD, G1 Therapeutic, Pierre Fabre Oncology, Servier, Roche, Daiichi and Gilead. F.D. received research funding and served as a speaker/advisor (compensated by the hospital) for Roche, Novartis, Lilly, Pfizer, Eisai, MSD and AstraZeneca. C.L.-P. received research funding and served as a speaker/advisor (compensated by the hospital) for AstraZeneca, Roche and Pfizer. A.G. received research funding and served as a speaker/advisor (compensated by the hospital) for AstraZeneca, Pfizer, Novartis, Roche and MSD. M.-A.M.R. received research funding and served as a speaker/advisor for Pfizer, Novartis, Lilly, Roche, MSD and Myriad. W.J. received research funding and served as a speaker/advisor for AstraZeneca, BMS, Daiichi Sankyo, Eisai, Lilly France, MSD, Novartis, Pfizer and Roche. B.Y. received research funding and served as a speaker/advisor for MSD, AstraZeneca, GSK-TESARO, BAYER, Roche-Genentech, ECS Progastrine, Novartis, LEK, Amgen, Clovis Oncology, Merck Serono, BMS, SEAGEN and Myriad. P.B. served as a speaker/advisor for Roche, BMS, IPSEN, MSD, Bayer, Amgen, Esai, Janssen Cilag, Pfizer, Novartis and Astellas and received honoraria from Seagen. N.H. received research funding from Novartis and Pfizer, and served as a consultant for AstraZeneca, Roche, Novartis and Pfizer. N.I. received research funding and served as a speaker/advisor (compensated by the hospital) for Ipsen and Transgene. J.-C.T. received research funding and served as a speaker/advisor (compensated by the hospital) for Pfizer and AstraZeneca. J.-M.F. received research funding and served as a speaker/advisor for Pfizer and Esai. T.F. consulted for Cellectis (compensated by the hospital). The following authors declared no competing interests: F. Mosele, L.L., G.E., A. Mege, F.C., M.-P.S., I.B., E.R., I.S., V.A., S.B., A. Morel, A.T.-D., A.L., H.B., M.J., A.J., L.L.C., M.K., P.G. and N.S. C.L. participated in advisory boards for MSD, BMS, Merck Serono, GSK, AstraZeneca, Nanobiotix, Roche, Rakuten, Seattle Genetics and Celgene. F. Montemurro received consultancy fees from Roche, AstraZeneca, Daiichi Sankyo, SeaGen, MSD, Eli Lilly, Pierre Fabre and Novartis, and travel grants from Roche.

Additional information

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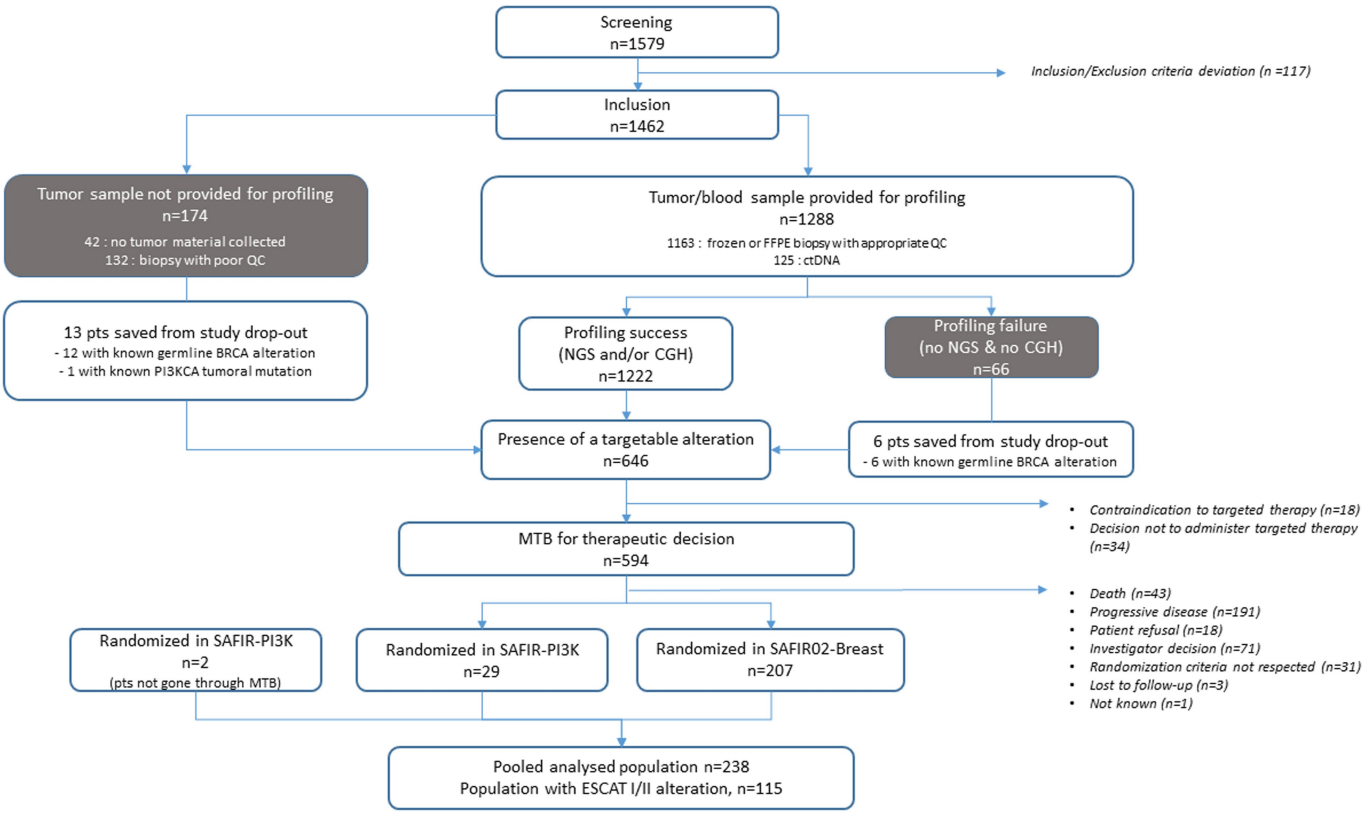
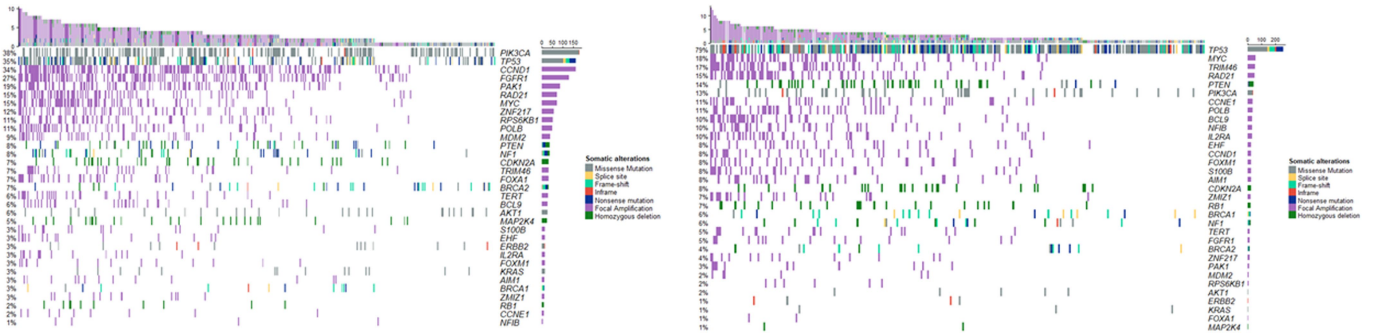
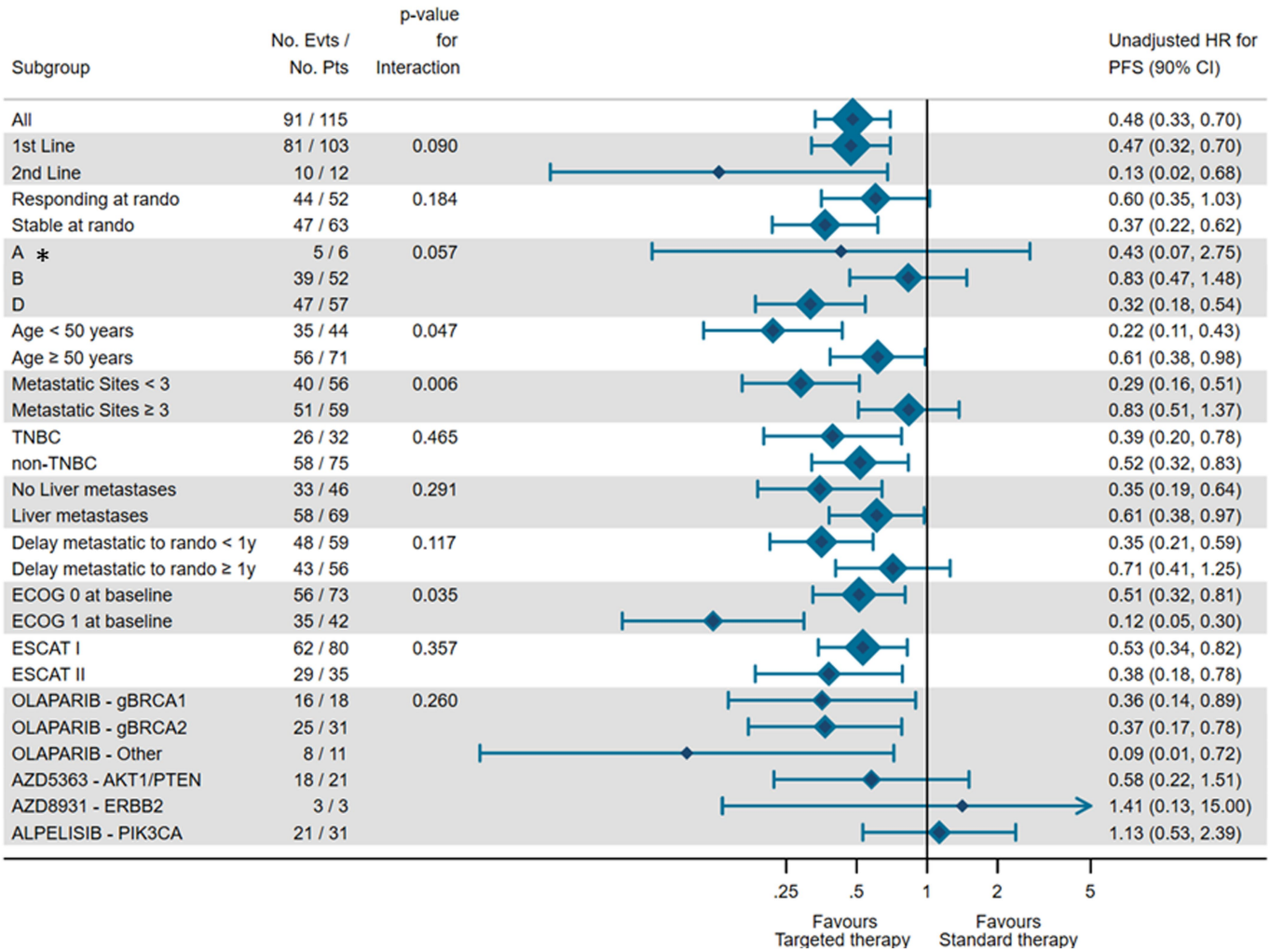


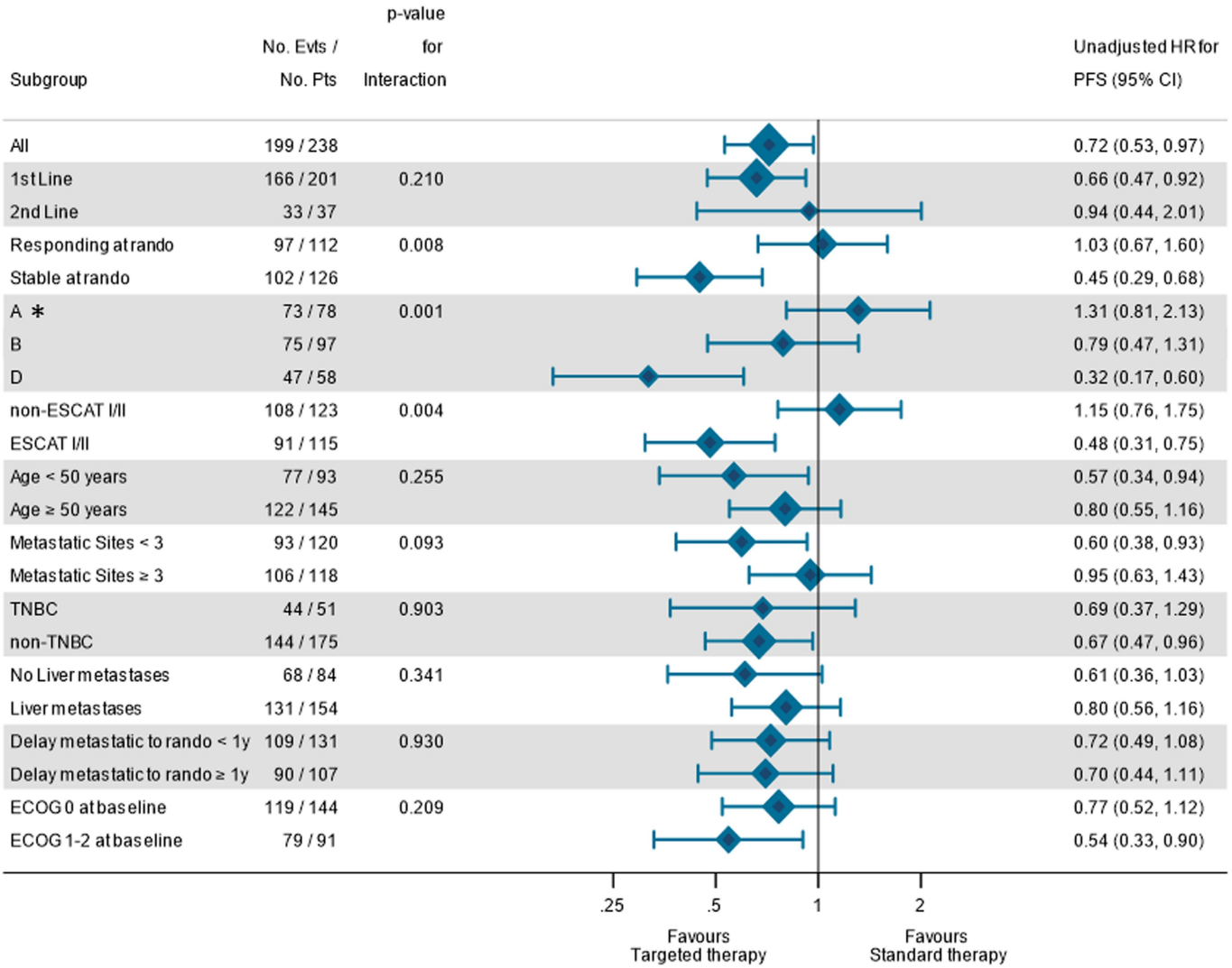
Extended Data Fig. 1 | CONSORT diagram of the trial.



Extended Data Fig. 2 | Genomic alterations identification in patients with HR+/Her2- (left panel) or TNBC (right panel). The analysis focuses on the 50 genes that were included in the first panel and on the copy number analyses.



Extended Data Fig. 3 | Subgroup analysis regarding efficacy of targeted therapy on Progression free survival, in patients presenting an ESCAT I/II alteration. The figure reports unadjusted Hazard Ratio (diamonds) and 90% confidence intervals (error bars) estimated using a Cox proportional hazard model in each subgroup for progression or death according to clinical and biological variables. P-value for interaction between treatment arm and each variable was estimated using a Cox proportional hazard model fitted with the treatment arm, the variable and an interaction term between treatment arm and variable. All statistical tests were two sided. No adjustment was made for multiple comparisons. *: A: tyrosine kinase, B: PI3K/mTOR pathway, C: MEK pathway, D: DNA repair.



Extended Data Fig. 4 | Subgroup analysis regarding efficacy of targeted therapy on progression free survival, in the intent-to-treat population. The figure reports unadjusted Hazard Ratio (diamonds) and 95% confidence intervals (error bars) estimated using a Cox proportional hazard model in each subgroup for progression or death according to clinical and biological variables. P-value for interaction between treatment arm and each variable was estimated using a Cox proportional hazard model fitted with the treatment arm, the variable and an interaction term between treatment arm and variable. All statistical tests were two sided. No adjustment was made for multiple comparisons. *: A: tyrosine kinase, B: PI3K/mTOR pathway, C: MEK pathway, D: DNA repair.

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Software and code

Policy information about availability of computer code

Data collection	data collection was performed using CSOnline module of Ennov Clinical
Data analysis	Statistical analyses were performed using Stata 16 software (StataCorp, Texas, US). Loss-of-heterozygosity state, homologous recombination deficiency score were performed using rCGH, EaCoN (R packages) and HRDetect respectively. R version 4.1.1 was used

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Genomic data and modalities for access are available at EGAS00001005584 and https://nextcloud.gustaveroussy.fr/s/JXLt7taZs8EtBF7

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The primary endpoint of the SAFIR 02 breast study is progression free survival in a pooled analysis of substudy 1 and a sample of patients from the SAFIR-PI3K protocol. In the ESCAT I/II population, the main objective is to detect a hazard ratio of 0.51, which is defined as clinically relevant. This hypothesis corresponds to increase the median Progression Free Survival from 3 months (Arm B1: "Maintenance therapy arm") to 5.88 months (Arm A1: "Targeted arm"). A total of 85 events are necessary for 90% power to detect this difference if it is true using a two-sided logrank test at the 10% level of significance and a 2:1 randomization (arm A1:arm B1). In the Intent to treat population, the main objective is to detect a hazard ratio of 0.66, which is defined as clinically relevant. This hypothesis corresponds to increase the median progression free survival from 3 months (Arm B1: "non genomic arm") to 4.54 months (Arm A1: "Genomic Arm"). A total of 205 events are necessary for 80 % power to detect this difference if it is true using a two–sided logrank test at the 5 % level of significance and a 2:1 randomisation (arm A1: arm B1). Based on an estimated accrual rate of approximately 4 patients per month for the randomization of 240 patients we can expect to see this number of events 57 months after the start of the study. With the inclusion of 240 patients it was expected that at least 110 patients will fall in the ESCAT I/II categories. In order to achieve these objectives, 1460 patients will be enrolled in the molecular screening phase over a 6 years period. Based on the rates of progression-free survival observed during the chemotherapy phase and the number of genomic abnormalities identified in SAFIR01 (Andre, Lancet Oncol 2014), we estimate that around 240 patients will actually receive the treatment.
Data exclusions	In the clinical trial, no data were excluded. Annex 1 and 2 includes all analyses that have been done by the statistician. For the analyses of genes with different copy numbers between early stage and metastases, tumor samples that 1) were ctDNA; 2) failed during the processing; 3) had a flat (low-dynamic) CGH profile; 4) were considered as too noisy (a threshold of 1500 segments by profile was used to considered a sample as noisy) or 5) showed ERBB2 amplifications were excluded
Replication	Since the paper reports a prospective clinical trial, there is no attempt to replicate the finding in the same paper. Nevertheless, the primary objective of the study was predefined, and thus limits the risk of non replication
Randomization	The trial was a randomized trial. The primary (PFS) and secondary (OS) endpoints were analyzed by Cox model adjusted for stratification factors (line of chemotherapy, disease status at randomization, group of genomic alterations)
Blinding	Blinding was not possible because some drugs were IV in the control arm and all drugs were oral in the experimental arm. Also, the safety profile of the drugs was very different, meaning a blinding would not be effective

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	The patients presented a metastatic breast cancer that did not overexpressed Her2. For cancers with expression of hormone receptors, the patients were included if they presented a resistance to endocrine therapy. No more than one previous line of chemotherapy was allowed in the metastatic setting. Stratification factors included line of chemotherapy, disease status at

	the time of randomization, group of genomic alteration. 77% of patients presented a Hormone receptor positive breast cancer. 59% of patients previously received a chemotherapy in the metastatic setting. 49% of the patients had less than three metastatic sites. 64% of patients had liver metastases. 47% of patients had an objective response to chemotherapy at the time of randomization
Recruitment	patients were reruited by their oncologists. We did not identify self-selectoin bias or other bias that could impact the results
Ethics oversight	All patients who entered in the randomized part of SAFIR02-BREAST and SAFIR-PI3K signed informed consent. The SAFIR02-BREAST trial was approved by the French ethics committee, CPP - Ile de France 2, on December 13th 2013 (ref 2013-09-07) and the French health authorities, ANSM, on January 14th2014 (ref 2013-001652-36). The SAFIR-PI3K trial was approved by the French ethics committee, CPP –Sud Ouest et Outre Mer2, on July7th 2017 (ref 2-17-21) and the French health authorities, ANSM, on July 19th 2017 (ref 2017-000154-19). SAFIR-PI3K was introduced in SAFIR02-BREAST trial in 2017 and was approved by the French ethics committee, CPP - Ile de France 2, on September17th 2017 (ref 2013-09-07 MS10) and French health authorities on December 22th 2017, ANSM (ref 2013-001652-36 MS10). An Independent Data Monitoring Committee (IDMC) that included 6 members monitored the study every 6 months.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT02299999 ,NCT03386162
Study protocol	Annex 3,4,5
Data collection	Trials included patients between 2014 and 2021. Data were captured by clinical research assistants in the centers that participated to the trial. Trial monitoring was done on a regular basis by the Sponsor UNICANCER.
Outcomes	Progression-Free Survival (PFS) is defined as the time from randomization to the first documented progression of disease (assessed via RECIST 1.1 or clinical progression) or death due to any cause. Patients still alive at the time of analysis without documented progression (including lost to follow-up) is censored at the last tumor assessment date. Overall Survival (OS) is defined as the time from randomization to death due to any cause. Patients still alive at the time of analysis (including lost to follow-up) is censored at the last known alive date. More details are available in the statistical analysis plan.