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Durvalumab compared to maintenance chemotherapy in metastatic breast cancer: the randomized phase II SAFIR02-BREAST IMMUNO trial

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The impact of single-agent antibodies against programmed death-ligand 1 (PD-L1) as maintenance therapy is unknown in patients with metastatic breast cancer. The SAFIR02-BREAST IMMUNO substudy included patients with human epidermal growth factor receptor type 2 (Her2)-negative metastatic breast cancer whose disease did not progress after six to eight cycles of chemotherapy. Patients ($n = 199$) were randomized to either durvalumab (10 mg kg^{-1} every 2 weeks) or maintenance chemotherapy. In the overall population, durvalumab did not improve progression-free survival (adjusted hazard ratio (HR): 1.40, 95% confidence interval (CI): 1.00–1.96; $P = 0.047$) or overall survival (OS; adjusted HR: 0.84, 95% CI: 0.54–1.29; $P = 0.423$). In an exploratory subgroup analysis, durvalumab improved OS in patients with triple-negative breast cancer (TNBC; $n = 82$; HR: 0.54, 95% CI: 0.30–0.97, $P = 0.0377$). Exploratory analysis showed that the HR of death was 0.37 (95% CI: 0.12–1.13) for patients with PD-L1$^+$ TNBC ($n = 32$) and 0.49 (95% CI: 0.18–1.34) for those with PD-L1$^-$ TNBC ($n = 29$). In patients with TNBC, exploratory analyses showed that the HR for durvalumab efficacy (OS) was 0.18 (95% CI: 0.05–0.71; log-rank test, $P = 0.0059$) in patients with CD274 gain/amplification ($n = 23$) and 1.12 (95% CI: 0.42–2.99; log-rank test, $P = 0.8139$) in patients with CD274 normal/loss ($n = 32$). Tumor infiltration by lymphocytes (CD8, FoxP3 and CD103 expressions) and homologous recombination deficiency did not predict sensitivity to durvalumab in exploratory analyses. This latter finding should be interpreted with caution since only one patient presented a germline *BRCA* mutation. The present study provides a rationale to evaluate single-agent durvalumab in maintenance therapy in patients with TNBC. Exploratory analyses identified CD274 amplification as a potential biomarker of sensitivity. Maintenance chemotherapy was more effective than durvalumab in patients with hormone receptor-positive and Her2-negative disease.

Breast cancer includes three major molecular subtypes, namely Her2-overexpressing, hormone receptor-positive (HR$^+$) and TNBC. HR$^+$ and Her2-overexpressing metastatic breast cancers (mBCs)

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have been transformed by targeted therapies (endocrine therapy, CDK4/6 inhibitors and Her2 inhibitors)[1]. Anti-PD-L1 antibodies inhibit immunosuppressive signaling mediated by PD1. Several phase II trials have suggested that a subset of patients with mBC could derive benefit from these immunotherapies[2]. More recently, a phase III trial combining chemotherapy with an anti-PD-L1 antibody reported moderate efficacy on progression-free survival (PFS) in patients with metastatic TNBC[3]. Several questions remain. First, the efficacy of single-agent anti-PD-L1 antibodies as maintenance therapy is unknown in metastatic TNBC patients. Second, while a small phase II randomized trial suggested that pembrolizumab could be effective in the neoadjuvant setting[4], there is no randomized trial reporting efficacy of anti-PD-L1 antibodies in patients with HR$^+$ mBC. Third, no biomarker exists so far to predict which patient will benefit from anti-PD-L1 antibodies.

Several studies have reported predictive value for tumor-infiltrating lymphocytes, PD-L1 and tumor mutational burden in TNBC. In a phase I trial that included 116 patients with metastatic TNBC, PD-L1 expression and infiltration of immune cells was associated with better response rates and OS[5]. In the TONIC trial[6], which tested nivolumab after a short induction chemotherapy, tumor infiltration by lymphocytes was associated with better objective response rates. Similar findings were reported by Loi et al.[7] using samples from KN-086, a single-arm phase II trial testing pembrolizumab in patients with metastatic TNBC. Finally, the predictive value of tumor mutational burden for the efficacy of anti-PD1 antibodies in breast cancer is still a matter of controversy[6,8,9]. Little is known about predictive biomarkers when anti-PD-L1 antibodies are given in the maintenance setting. Herein we report the results and biomarker analyses of the phase II randomized trial SAFIR02-BREAST IMMUNO, which compared an anti-PD-L1 antibody (durvalumab) to maintenance chemotherapy in patients with Her2-negative mBC.

One hundred and ninety-nine patients from the SAFIR02-BREAST trial, without progressive disease after initial chemotherapy, were randomized (at a 2:1 ratio) to either durvalumab (10 mg kg^{-1} every 2 weeks until progressive disease or toxicity; $n=131$) or maintenance chemotherapy ($n=68$; Extended Data Fig. 1). Patients were selected for not presenting a targetable molecular alteration from the SAFIR02-BREAST trial. In addition, in 14 patients, a somatic genomic alteration was detected in the SAFIR02-BREAST trial, but the investigator considered that the proposed targeted therapy was not matched (*PIK3CA* mutation before alpelisib ($n=7$), *NF1* mutation ($n=2$) or other alteration ($n=5$)). The CONSORT (Consolidated Standards of Reporting Trials) diagram is reported in Extended Data Fig. 2, and patient characteristics are reported in Supplementary Table 1. Eighty-two (43%) patients had TNBC and 108 (56%) had HR$^+$/Her2$^-$ breast cancer. The estrogen receptor (ER), progesterone receptor (PR) and Her2 statuses, using the primary tumor sample, were used to defined TNBC status for analyses. One hundred and seventy-nine (90%) patients were randomized after first-line induction chemotherapy and 81 (41%) presented a tumor response after this induction chemotherapy. Approximately 94% of patients received six to eight cycles of induction chemotherapy.

The median number of cycles was seven (range 1 to 49) in the durvalumab arm and four (range 1 to 35) in the maintenance chemotherapy arm. The most commonly used regimen in the maintenance arm was bevacizumab alone or in combination in patients

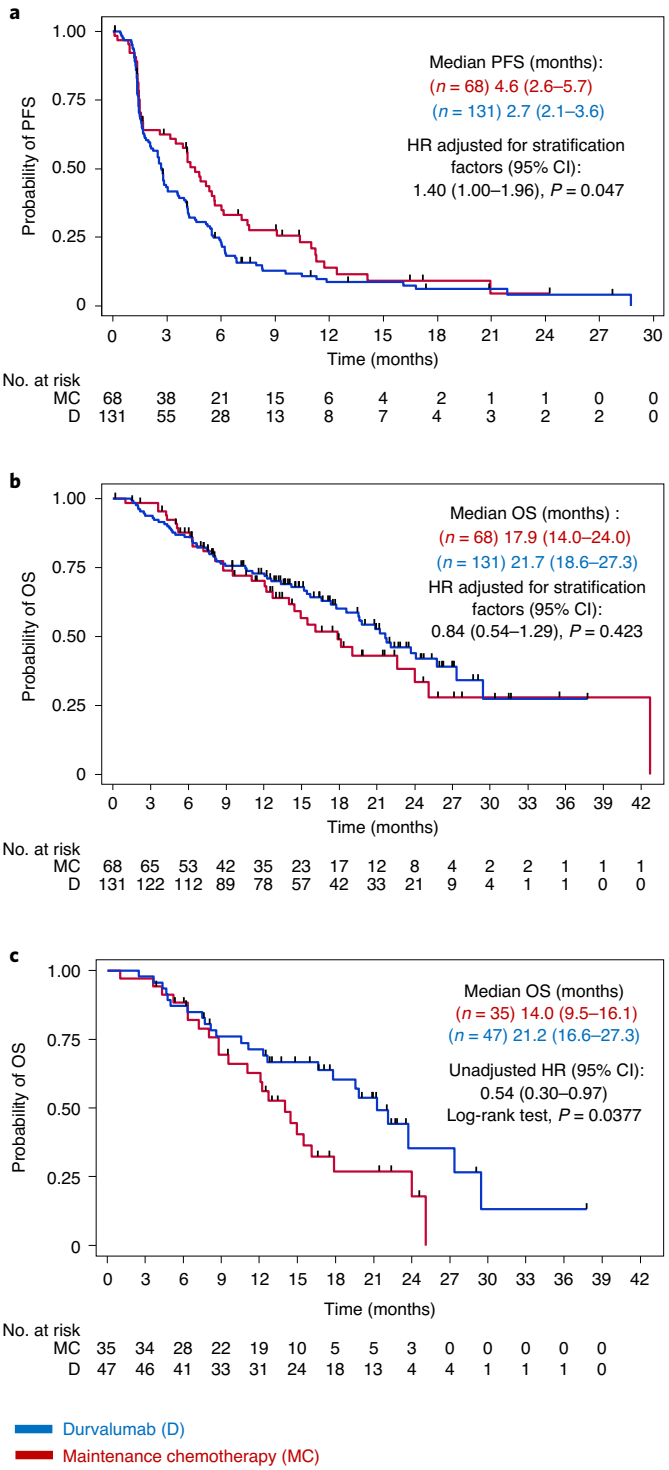


Fig. 1 | Kaplan–Meier plots of progression-free and overall survival. a, Efficacy of durvalumab ($n=131$) compared to maintenance chemotherapy ($n=68$) on PFS in the overall population of SAFIR02-BREAST IMMUNO. The HR and *P* value were derived from a Cox proportional hazard model adjusted for stratification factors: 1.40 (95% CI: 1.00–1.96); $P=0.047$. Two-sided statistical test with no adjustment for multiple comparisons. **b**, The efficacy of durvalumab ($n=131$) compared to maintenance chemotherapy ($n=68$) on OS in the overall population of SAFIR02-BREAST IMMUNO. The HR and *P* value were derived from a Cox proportional hazard model adjusted for stratification factors: 0.84 (95% CI: 0.54–1.29); $P=0.423$. Two-sided statistical test with no adjustment for multiple comparisons. **c**, The efficacy of durvalumab ($n=47$) compared to maintenance chemotherapy ($n=35$) on OS in patients presenting a TNBC. The unadjusted HR from the Cox proportional hazard model was 0.54 (95% CI: 0.30–0.97), and the *P* value from a two-sided log-rank test was 0.0377. No adjustment was made for multiple comparisons.

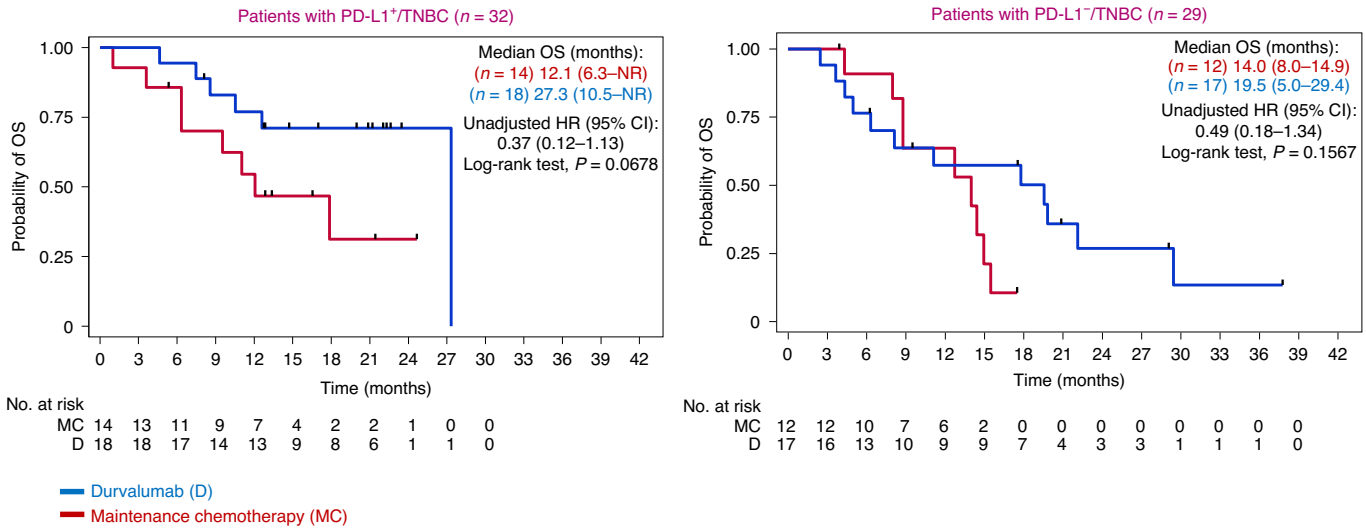


Fig. 2 | Kaplan–Meier plot of overall survival in TNBC according to PD-L1 expression. The efficacy of durvalumab on OS according to PD-L1 expression in patients presenting with TNBC. Unadjusted HR (95% CI) from a Cox proportional hazard model was 0.37 (0.12–1.13) for patients with PD-L1$^+$ TNBC ($n=32$) and 0.49 (0.18–1.34) for patients with PD-L1$^-$ TNBC ($n=29$). *P* values were calculated using a two-sided log-rank test. No adjustment was made for multiple comparisons. NR, not reached.

with TNBC ($n=12$; 34%), and paclitaxel alone or in combination in patients without TNBC ($n=11$; 34%). Ten patients did not receive maintenance chemotherapy after induction chemotherapy. The regimen given in the control arm is reported in Supplementary Table 1. In total, 121 (92%) patients treated with durvalumab and 52 (90%) treated with chemotherapy permanently discontinued treatment. The reason for discontinuation was disease progression in 150 patients (87%). Adverse events are reported in Supplementary Tables 2 and 3 and are consistent with previous reports[10,11].

In the intention-to-treat population, the median follow-up time was 19.7 (95% CI: 16.5–22.3) months. At analysis, 171 (86%) patients had progressive disease or had died. The median PFS was 2.7 (95% CI: 2.1–3.6) and 4.6 (95% CI: 2.6–5.7) months in the durvalumab and maintenance chemotherapy arms, respectively (HR adjusted for stratification factors: 1.40 (95% CI: 1.00–1.96); $P=0.047$; Fig. 1a). No clinical subgroup derived benefit from durvalumab maintenance in terms of PFS (Extended Data Fig. 3). The HR for durvalumab was 0.87 (95% CI: 0.54–1.42) in patients with TNBC ($n=82$) and 2.08 (95% CI: 1.28–3.40) in those without TNBC ($n=110$). The HR for durvalumab was 1.08 (95% CI: 0.72–1.63) in patients with stable disease at randomization ($n=118$) and 1.89 (95% CI: 1.09–3.28) in those with a response ($n=81$). We then assessed the efficacy of durvalumab in terms of OS. In the overall population, no significant OS benefit was observed. The median OS was 21.7 (95% CI: 18.6–27.3) months in the durvalumab arm ($n=131$) and 17.9 (95% CI: 14.0–24.0) months in the chemotherapy arms ($n=68$; HR adjusted for stratification factors: 0.84, 95% CI: 0.54–1.29, $P=0.423$; Fig. 1b). More than two metastatic sites (interaction, $P=0.098$), an interval between diagnosis of metastases to randomization below 1 year (interaction, $P=0.055$) and TNBC subtypes (interaction, $P=0.083$) were associated with an increased benefit of durvalumab on OS (Extended Data Fig. 4). In an exploratory analysis, in the TNBC subgroup ($n=82$), the median OS was 21.2 (95% CI: 16.6–27.3) months with durvalumab compared with 14.0 (95% CI: 9.5–16.1) months with maintenance chemotherapy (HR: 0.54, 95% CI: 0.30–0.97; log-rank test, $P=0.0377$; Fig. 1c). We further explored candidate predictive biomarkers to identify patients who could benefit from single-agent anti-PD-L1 antibodies in the maintenance setting. These biomarker analyses should be considered exploratory and be interpreted with caution given the retrospective nature and the small number of samples.

We first evaluated the predictive value of PD-L1 expression. PD-L1 protein expression was assessed by immunohistochemistry (IHC) using SP142 antibody, as previously reported[3]. PD-L1 expression was determined using a sample from a metastatic lesion obtained within 1 year before patient inclusion. Of 133 samples, 44 expressed PD-L1 (≥1% of immune cells stained). In patients with PD-L1-expressed tumors ($n=44$), the median OS was 25.8 (95% CI: 15.4–not reached) months in the durvalumab arm and 12.1 (95% CI: 6.3–not reached) months in the maintenance chemotherapy arm (HR: 0.42, 95% CI: 0.17–1.05; log-rank test, $P=0.055$). Nevertheless, this predictive value is probably related to an enrichment of PD-L1 expression in TNBC. Indeed, PD-L1 expression was found in 52% of patients with TNBC and 15% of those without TNBC. HRs for death were 0.37 (95% CI: 0.12–1.13; Fig. 2) for patients with PD-L1$^+$ TNBC ($n=32$) and 0.49 (95% CI: 0.18–1.34; Fig. 2) for those with PD-L1$^-$ TNBC ($n=29$). The number of patients with the PD-L1$^+$ non-TNBC subtype was too low to explore efficacy in this group ($n=10$). CD8, CD103 and FoxP3 expressions, alone and combined, did not predict durvalumab efficacy in the overall population, nor in patients with TNBC (Supplementary Table 4). Tumor-infiltrating lymphocytes were not predictive of the efficacy of durvalumab (continuous variable, interaction test: $P=0.856$ for PFS and $P=0.177$ for OS).

We then investigated whether copy number alterations in the tumor cells could predict the efficacy of durvalumab. We first evaluated the predictive value of homologous recombination deficiency (HRD). HRD was assessed in 120 samples using HRDScore (Methods). A cutoff of 42 chromosome breaks was used to classify patients into HRD-low or HRD-high subgroups (Methods). The correlations between HRD and clinical characteristics are reported in the statistical report (Supplementary information). HRD did not predict efficacy of durvalumab in terms of OS, neither as binary variable (*P* value for interaction = 0.246) nor as continuous variable (*P* value for interaction = 0.565). When the analysis was limited to patients with TNBC, HRs for durvalumab efficacy in terms of OS were 0.27 (95% CI: 0.07–1.10) and 0.71 (95% CI: 0.26–1.89) in patients with HRD-low ($n=21$) and HRD-high ($n=31$), respectively. Survival curves are reported in Fig. 3a. We then assessed the

predictive value of *CD274* gain/amplifications. *CD274* encodes the CD274 molecule alias PD-L1. *CD274* was gained/amplified in 30 of 126 assessable samples (24%). An illustration of a CD274 amplification is shown in Fig. 3b. *CD274* gains/amplifications were observed in 23 of 55 TNBC samples (42%). In contrast, only 7 of 67 patients without TNBC (10%) presented *CD274* gene gains/amplifications. *CD274* gain/amplification was associated with PD-L1 expression by cancer cells. PD-L1 expression on cancer cells was observed in 4 of 26 patients with *CD274* gain/amplification compared to 2 of 72 without *CD274* gain/amplification ($P=0.04$). Conversely, no correlation was observed between *CD274* gain/amplification and PD-L1 expression by immune cells ($P=0.41$). *CD274* gain/amplification predicted efficacy of single-agent durvalumab in terms of OS (interaction test, $P=0.002$). The interaction between *CD274* gain/amplification and treatment remained significant after adjustment for PD-L1 IHC on immune cells in the overall population (interaction test, $P<0.001$) and in the TNBC subgroup (interaction test, $P=0.001$). Patients with *CD274*-amplified tumors were highly sensitive to durvalumab (HR = 0.17, 95% CI: 0.05–0.55; log-rank test, $P=0.0009$). Since *CD274* gain/amplification was strongly associated with TNBC status, we assessed the benefit of durvalumab according to *CD274* gene copy numbers and TNBC status. In patients with TNBC, the HR for durvalumab efficacy in terms of OS was 0.18 (95% CI: 0.05–0.71; log-rank test, $P=0.0059$) in patients with *CD274* gain/amplification ($n=23$) and 1.12 (95% CI: 0.42–2.99; log-rank test, $P=0.8139$) in patients *CD274* normal/loss ($n=32$). Survival curves are shown in Fig. 3c. Of the seven patients who presented a *CD274* gain/amplification and non-TNBC subtype, six received durvalumab and five were alive after 15, 16, 19, 24 and 26 months.

While an unusual number of patients with metastatic TNBC live long after treatment with anti-PD-L1 antibody[2,12], evidence from randomized trials is currently lacking. IMpassion130 (ref. [3]) reported a modest improvement in PFS and, because of hierarchical testing, could not test for OS improvement. IMpassion131 could not detect a benefit for atezolizumab in combination with paclitaxel[13], and KN355 showed a marginal benefit on PFS[14]. Such weak benefit could be explained by the fact that each of these trials combined frontline anti-PD-L1 or anti-PD1 antibodies and chemotherapy. Indeed, combining anti-PD-L1 or PD1 antibodies with chemotherapy could decrease their efficacy through the use of steroids (IMpassion131) or chemo-induced immune suppression. Also, immunotherapeutics could be more effective after the bulk of disease has been decreased. Finally, randomization at the initiation of chemotherapy could improve the performance of the control arm. All these data provided a rationale to test durvalumab in the maintenance setting, rather than upfront. The PACIFIC[10] and JAVELIN Bladder 100 (ref. [15]) randomized trials have previously shown that this approach is effective in non-small cell lung cancer and urothelial tumors. SAFIR02-BREAST IMMUNO reports consistent data with these trials, in patients with metastatic TNBC. Biological studies also support this hypothesis. Indeed, it has been reported by several groups that cytotoxic chemotherapy attracts lymphocytes on the tumor bed and could therefore operate as a switch from immune-deficient to inflamed cancers[16,17]. Finally, selecting patients who are sensitive to chemotherapy could have enriched SAFIR02-BREAST IMMUNO in patients who are more sensitive to durvalumab. Indeed, several studies performed in the neoadjuvant setting have reported that lymphocytic infiltration or PD-L1 expression could be predictive for the efficacy of chemotherapy in patients with TNBC[18].

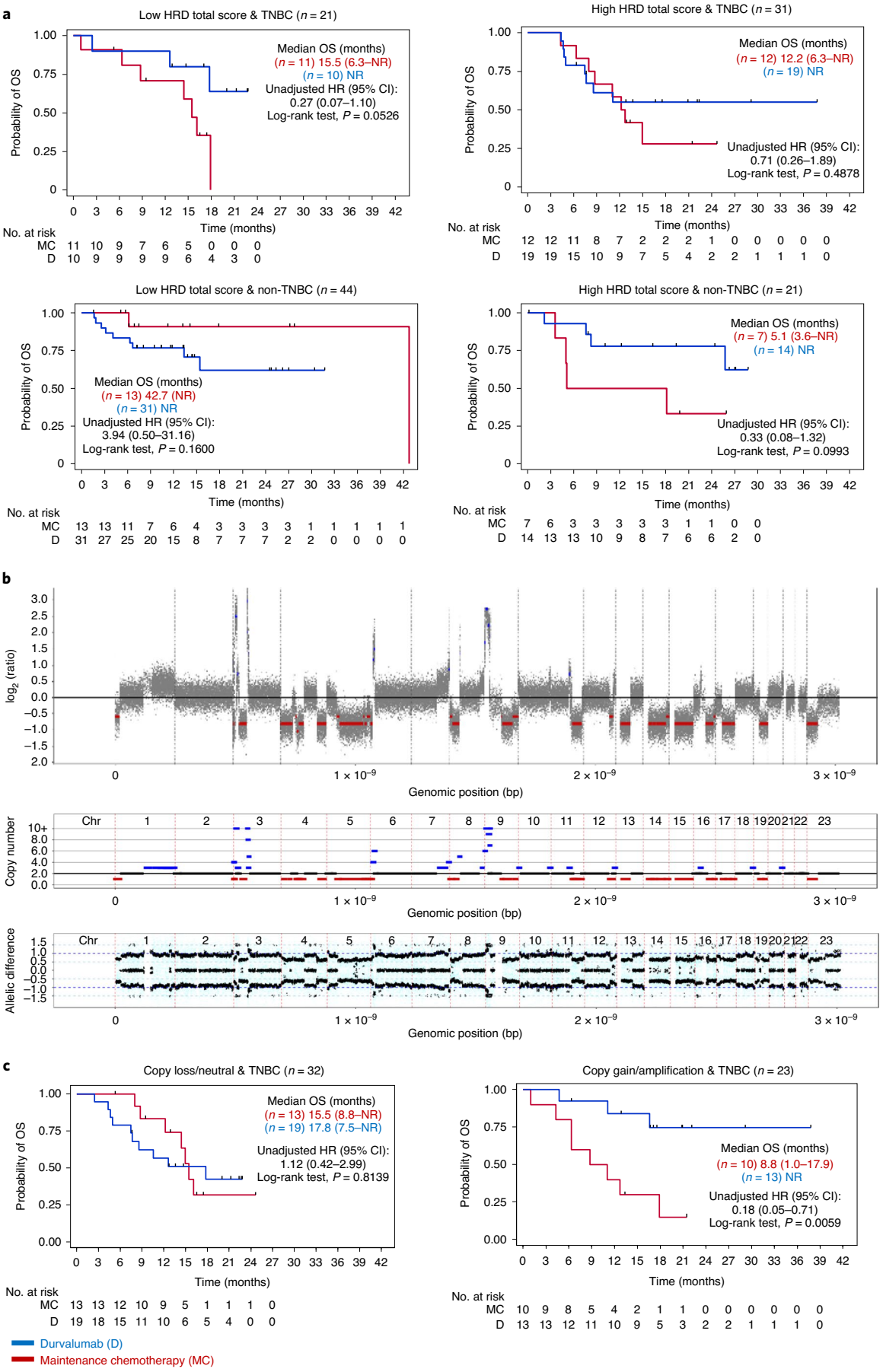
The IMpassion130 study results suggest that PD-L1 expression could predict the benefit of atezolizumab in patients with metastatic TNBC[3]. In SAFIR02-BREAST IMMUNO, while PD-L1 expression was predictive in the overall population, it was not predictive in patients with TNBC. This could be explained by the exposure to chemotherapy between the biopsy and the start of immunotherapeutics[19,20]. A previous phase II study suggested that immunotherapy could have minor activity in patients with HR^+ mBC[21]. In the current study, durvalumab was inferior to maintenance chemotherapy in this group of patients. Nevertheless, because of the small sample size, we cannot exclude a benefit in this subgroup of patients. For example, the number of patients with PD-$L1^+$ expression was too small ($n=10$) to test its predictive value.

CD274 copy gain or amplification were associated with high sensitivity to durvalumab. The finding that a subset of tumors presents CD274 amplifications has been reported in several studies[22,23]. Nevertheless, its correlation with outcome and clinical characteristics is still unknown. Goodman et al.[22] reported that *CD274* amplifications, defined by six or more copies per cell, were present in only a small subset of primary tumor samples (1.9% for breast cancer) and were associated with PD-L1 expression by tumor cells. A preliminary analysis suggested that *CD274* amplification could identify patients who are sensitive to anti-PD1 antibodies (6 of 9 responders). Further studies are needed to validate these findings and to determine whether *CD274* gain/amplification causes PD1-mediated local immune suppression.

In the present study, T cell infiltration and HRD did not predict the efficacy of anti-PD-L1 antibodies. As for PD-L1 expression, the observation that T cell infiltration does not predictive efficacy should be taken with caution since patients received six to eight cycles of chemotherapy between biopsy and the start of durvalumab. Finally, it is important to acknowledge that most of the patients with *BRCA1/2* or *PALB2* germline mutations were excluded from the SAFIR02-BREAST IMMUNO trial. Thus, the finding that HRD did not predict sensitivity to durvalumab should not be extrapolated to these patients.

The patient population included in the current trial is not representative of the whole breast cancer population, and this could have contributed to the large benefit observed in patients with metastatic TNBC. Indeed, patients with an actionable genomic alteration were mostly driven to arm targeted therapies of SAFIR02-BREAST (Extended Data Fig. 1), meaning that patients with *PTEN* deletion and *BRCA* mutations were not driven to SAFIR02-BREAST IMMUNO. While the number of *PTEN* deletions was small ($n=4$), overall, 24 patients with a *BRCA* mutation and metastatic TNBC were driven to arm targeted therapies of SAFIR02-BREAST. Consequently, only one patient included in SAFIR02-IMMUNO

Fig. 3 | Kaplan–Meier plot of overall survival according to genomic markers. a, Efficacy of durvalumab on OS according to HRD and TNBC status. The HRD score was determined as previously reported[25]. HRD score measures the frequency of large-scale loss of heterozygosity (LOH), telomeric allelic imbalance and large-scale transition events. A cutoff of 42 was selected to define an HRD tumor, as previously reported[26,27]. Unadjusted HRs (95% CI) for durvalumab efficacy from the Cox proportional hazard model were 0.27 (0.07–1.10) for patients with a HRD-low/TNBC subtype ($n=21$), 0.71 (0.26–1.89) for patients with HRD-high/TNBC subtype ($n=31$), 3.94 (0.50–31.16) for patients with HRD-low/non-TNBC subtype ($n=44$) and 0.33 (0.08–1.32) for patients with HRD-high/non-TNBC subtype ($n=21$). *P* values were calculated using two-sided log-rank test. No adjustment was made for multiple comparisons. **b**, Illustration of CD274 amplification (PD-L1). **c**, Efficacy of durvalumab on OS according to CD274 amplification, in patients with TNBC. The unadjusted HR for durvalumab efficacy from the Cox proportional hazard model was 1.12 (95% CI: 0.42–2.99) in patients with CD274 normal/loss ($n=32$) and 0.18 (0.05–0.71) in patients with CD274 gain/amplification ($n=23$). *P* values were calculated using two-sided log-rank test. No adjustment was made for multiple comparisons.


a
Low HRD total score & TNBC (n = 21)
Median OS (months)
(n = 11) 15.5 (6.3–NR)
(n = 10) NR
Unadjusted HR (95% CI):
0.27 (0.07–1.10)
Log-rank test, P = 0.0526
Probability of OS
Time (months)
No. at risk
MC 11 10 9 7 6 5 0 0 0
D 10 9 9 9 9 6 4 3 0
High HRD total score & TNBC (n = 31)
Median OS (months)
(n = 12) 12.2 (6.3–NR)
(n = 19) NR
Unadjusted HR (95% CI):
0.71 (0.26–1.89)
Log-rank test, P = 0.4878
No. at risk
MC 12 12 11 8 7 2 2 2 1 0 0 0 0 0
D 19 19 15 10 9 7 5 4 2 2 1 1 1 0
Low HRD total score & non-TNBC (n = 44)
Median OS (months)
(n = 13) 42.7 (NR)
(n = 31) NR
Unadjusted HR (95% CI):
3.94 (0.50–31.16)
Log-rank test, P = 0.1600
No. at risk
MC 13 13 11 7 6 4 3 3 3 3 1 1 1 1
D 31 27 25 20 15 8 7 7 7 2 2 0 0 0 0
High HRD total score & non-TNBC (n = 21)
Median OS (months)
(n = 7) 5.1 (3.6–NR)
(n = 14) NR
Unadjusted HR (95% CI):
0.33 (0.08–1.32)
Log-rank test, P = 0.0993
No. at risk
MC 7 6 3 3 3 3 1 1 0 0
D 14 13 13 10 9 8 7 6 6 2 0
b
log2 (ratio)
Genomic position (bp)
Copy number
Chr
Allelic difference
c
Copy loss/neutral & TNBC (n = 32)
Median OS (months)
(n = 13) 15.5 (8.8–NR)
(n = 19) 17.8 (7.5–NR)
Unadjusted HR (95% CI):
1.12 (0.42–2.99)
Log-rank test, P = 0.8139
No. at risk
MC 13 13 12 10 9 5 1 1 1 0
D 19 18 15 11 10 6 5 4 0 0
Copy gain/amplification & TNBC (n = 23)
Median OS (months)
(n = 10) 8.8 (1.0–17.9)
(n = 13) NR
Unadjusted HR (95% CI):
0.18 (0.05–0.71)
Log-rank test, P = 0.0059
No. at risk
MC 10 9 8 5 4 2 1 1 0 0 0 0 0 0
D 13 13 12 11 10 9 5 3 2 2 1 1 1 0
Durvalumab (D)
Maintenance chemotherapy (MC)

presented a germline *BRCA1* mutation. In IMpassion130, HRs for OS were 0.85 and 0.87 in patients with a *BRCA* mutation who presented a PD-L1$^-$ and PD-L1$^+$ TNBC, respectively, higher than that observed in patients without a *BRCA* mutation (HR: 0.62, 95% CI: 0.43–0.91)[24].

In the present study, maintenance chemotherapy was associated with longer PFS as compared to durvalumab. A subgroup analysis suggested that single-agent durvalumab given as maintenance therapy could improve outcomes in patients with TNBC, irrespective of their PD-L1 status. These data provide a rationale to test single-agent durvalumab as maintenance therapy after induction chemotherapy in patients with TNBC. Further studies are needed to validate the observation that CD274 gain/amplification could define a group of patients with high sensitivity to anti-PD-L1 antibodies. In patients with HR$^+$/Her2$^-$ disease, maintenance chemotherapy was more effective than durvalumab.

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/s41591-020-01189-2.

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Methods

Trial design. SAFIR02-BREAST IMMUNO is a phase II interventional randomized open-label trial that compares durvalumab (10 mg kg^{-1} every 2 weeks until progressive disease or toxicity) with maintenance chemotherapy in patients who do not present an actionable genomic alteration. The first patient was randomized on 27 January 2016 and the last patient on 13 September 2019. The SAFIR02-BREAST IMMUNO substudy (NCT02299999) is part of the SAFIR02-BREAST trial as summarized in Extended data Fig. 1. The SAFIR02-BREAST trial includes patients who have mBC without Her2 overexpression and who had received no or one line of chemotherapy in the metastatic setting. Patients with ER$^+$ disease were eligible if they relapsed or presented a disease progression either during endocrine therapy or less than 12 months after the end of endocrine therapy in the adjuvant setting. In total, 106 of the 108 patients with HR$^+$/Her2$^-$ breast cancer received a previous endocrine therapy, including at least one line of endocrine therapy in the metastatic setting in 65 patients (60%). After obtaining informed consent, a biopsy was performed, except in patients who were biopsied within 1 year before inclusion and for whom stored samples were available. In case of a bone lesion or if biopsy was not feasible, a plasma sample was obtained before the third cycle of chemotherapy. After six to eight cycles of chemotherapy (or at least four in case of toxicity), patients who did not present a progressive disease and did not present an actionable alteration defined by SAFIR02-BREAST protocol were randomized in a 2:1 ratio to either durvalumab or maintenance chemotherapy. Randomization was stratified, by the minimization method, according to the line of chemotherapy (first-line versus second-line) and the tumor response (stable disease versus tumor response). Maintenance chemotherapy was defined as the continuation of the chemotherapy administered during the initial six to eight cycles. Only patients who presented an objective response or stable disease were eligible for randomization. Assessment of objective response or stable disease after induction chemotherapy was at the discretion of the investigator and not confirmed by a subsequent evaluation. The SAFIR02-BREAST trial was approved by the French ethics committee CPP Ile de France 2 on 13 December 2013 (2013-09-07) and the French health authorities ANSM on 14 January 2014 (2013-001652-36). SAFIR02-BREAST IMMUNO substudy was added to the SAFIR02-BREAST trial in 2015 and was approved by the French ethics committee CPP Ile de France 2 on 15 October 2015 (2013-09-07 MS3) and French health authorities ANSM on 15 September 2015 (2013-001652-36 MS3). Between 2016 and 2019, we conducted the SAFIR02-BREAST IMMUNO substudy at 22 study sites in France in accordance with the Declaration of Helsinki, current International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines and all applicable regulatory and ethical requirements. Patients signed informed consent for biopsy, randomization and use of their biological samples for research purposes. The trial was funded by the Foundation ARC, AstraZeneca (investigator-initiated study grant) and the Breast Cancer Research Foundation. The study protocol and statistical report and biomarkers are available as Supplementary Information.

Treatments and follow-up. Patients signed the informed consent for randomization and were treated either by intravenous durvalumab (10 mg kg^{-1} every 2 weeks) or maintenance chemotherapy until disease progression or toxicity. Maintenance chemotherapy was identical to that administered during the initial six to eight cycles. Switching chemotherapy regimen was not allowed per the study protocol. Patients who switched treatment after randomization were censored ($n=5$), except if the switch involved endocrine therapy in patients resistant to aromatase inhibitors or metronomic cyclophosphamide. Treatment efficacy was monitored by a computed tomography scan every 6 weeks during the initial 6 months after randomization to maintenance, and every 6 to 9 weeks after 6 months of maintenance. The Response Evaluation Criteria in Solid Tumors (v1.1) were used to assess response and progression[28]. Based on the recommendations from the Independent Data Monitoring Committee, clinical progressions were also included as events for the primary end point. Toxicity data were collected at each visit and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (v4.03).

Immunohistochemistry. ER, PR and Her2 statuses were determined locally. ER and PR thresholds for positivity were set at 1% of tumor cells. ER and PR determinations were assessed on the primary tumor samples. Her2 was defined according to ASCO/CAP guidelines[29]. The association between IHC subtypes and durvalumab efficacy was assessed based on samples obtained from primary tumors, because the number of missing values was too high for metastatic samples ($n=77$). PD-L1 staining was assessed using SP142 antibody (Ventana Medical Systems, 790–4860; final dilution 7 µg ml^{-1}) as previously described[2]. A cutoff of 1% of PD-L1-expressing immune cells was used to define positivity. PD-L1 expression was performed on metastatic tumor samples. To assess whether T cell infiltration predicted sensitivity to durvalumab, we used a panel that tested expression of CD8, CD103 (memory resident T cells) and FoxP3 (regulatory T cells). Multiplexed fluorescent IHC for T cells was performed in a Ventana Discovery Ultra autostainer. Sequential staining included anti-CD103 (clone EPR4166-2; Abcam, ab129202; 1:200 dilution), anti-cytokeratin (clones AE1/AE3; Dako, M351501-2; 1:100 dilution), anti-CD8 (clone SP16; Spring Bioscience, M3164; 1:100 dilution) and anti-FoxP3 (clone SP97; Spring Bioscience, M3974; 1:150 dilution). The technical parameters were optimized for tissue type and antibody. HRP-conjugated amplification systems Discovery UltraMap anti-rabbit HRP and anti-mouse HRP (Ventana) were coupled to the fluorophores Opal 520 (FITC), Opal 570 (Cy3), Opal 690 (Cy5) and Opal 620 (Texas Red), from the Opal Multiplex Tissue Staining (PerkinElmer) and combined with Vectra 3 microscope and inForm image analysis software (v2.4.6; PerkinElmer). The analysis of the regions of interest, selected from the multispectral fluorescent images, consisted of spectral unmixing, tissue segmentation of epithelial and stromal zones based on trainable feature-recognition algorithms, cell segmentation by nuclear DAPI signal and the assigned phenotypes to individual cells according to their biomarker expression. Depending on the phenotyping performed, which was verified in all cases, the data extracted from each image were exported for cell density assessments on the different compartments (intraepithelial, stromal and total area). Assessment of tumor-infiltrating lymphocytes was performed as previously reported[30].

Genomic testing. The tumor samples were qualified for further genomic testing if more than 30% of the cells in the biopsy sample were cancer cells for frozen core biopsies and between 10–30% for formalin-fixed paraffin-embedded (FFPE) tumor biopsies.

DNA was isolated from six FFPE tissue sections (each 6-µm thick). A seventh tissue section was stained with H&E. The tumor-rich areas were macro-dissected using a single-use blade, and the samples underwent proteinase K digestion in a rotating incubator at 56 °C for 3 d. DNA was extracted using the Nucleospin 8 Tissue kit (Macherey-Nagel).

Isolation of DNA from frozen core biopsies was performed using the AllPrep DNA/RNA Mini kit (Qiagen) according to the manufacturer's protocol. DNA was quantified using a Qubit 2.0 Fluorometer (Quant-iT dsDNA BR Assay Kit; Thermo Fisher Scientific), according to the manufacturer's instructions.

Copy number alterations were detected prospectively using the CytoScan HD Array Kit (Affymetrix, a Thermo Fisher Scientific company) for the fresh-frozen tissues and the OncoScan FFPE Assay Kit (Affymetrix) for the FFPE tissues. The OncoScan FFPE Assay Kit is a microarray designed specifically for use with degraded DNA, as is found in FFPE tissue. Both the OncoScan and the CytoScan arrays used single-nucleotide polymorphism probes to provide DNA copy number variations, according to the manufacturer's instructions.

Bioinformatic analysis. Copy number variations from CytoScan and OncoScan were defined using the R package rCGH[31] (v1.16.0, under R v3.6.3). Briefly, $\log_2$ relative ratios was calculated, a centralization of the profile set the baseline (two copies being the neutral level) from which copy number alterations were estimated. Break points in the $\log_2$ relative ratio continuity were identified by segmenting the profile. These segments were further used to determine a potential gain or loss, using the following scale: copy = 0: homozygous deletion; copy = 1: loss; copy = 2: copy neutral; $3 \leq$ copy ≤ 4: gain; and copy > 4: amplification. LOH state was defined using the R package EaCoN (v0.3.3), available at https://github.com/gustaveroussy/EaCoN/. The HRD score was determined as previously reported[25]. Based on the copy number alteration profile, HRDScore measures the frequency of large-scale LOH, telomeric allelic imbalance and large-scale transition events to define the HRD score. A cutoff of 42 was selected to define an HRD tumor, as previously reported[26,27].

Statistical analyses. The analysis of SAFIR02-BREAST IMMUNO is a secondary objective of the SAFIR02-BREAST trial. The primary and secondary end points of the SAFIR02-BREAST IMMUNO were PFS and OS, respectively. The definition of end points and prespecified exploratory subgroup analyses are detailed in the statistical analysis plan (Supplementary Information). No interim analysis for efficacy was planned. Additional post hoc exploratory analyses investigated lymphocytic infiltration using CD8, FoxP3 or CD103 expression assessed by multiplexed fluorescence IHC, tumor-infiltrating lymphocytes, genomic instability assessed by the number of chromosomes breaks, and genomic amplification of PD-L1 (CD274).

We determined that 166 instances of tumor progression or death would be needed in SAFIR02-BREAST IMMUNO (with an 80% power, a two-sided significance level of 0.05 and a 2:1 randomization ratio) to detect a HR for PFS of 0.63 (increase in median PFS from 3 months to 4.76 months). We estimated that the trial needed to enroll 190 patients to observe the number of events required.

The primary and secondary end points (PFS and OS) were analyzed in the intention-to-treat population with a Cox regression model, adjusted for the factors used for stratification of the randomization. For the subgroup analyses, treatment differences were tested using the log-rank test, and HRs were estimated with an unadjusted Cox proportional hazard model. The statistical significance of the interactions between treatment effects and subgroups/biomarkers were tested using a Cox proportional hazard model fitted with the biomarker/subgroup, an indicator for the treatment arm, and an interaction term between the treatment arm and subgroup/biomarker. Due to the exploratory nature of the subgroup and biomarker analysis, no adjustments were performed for multiple comparisons[32]. Statistical analysis was performed in Stata v16 (StataCorp).

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

Data availability

The datasets generated and analyzed (CEL files and HRD results) during the current study are available on synapse (https://www.synapse.org) under the ID syn22010057. Please read the wiki for more information about the contents of the syn22010057 folder (data access files, raw data, processed data).
Other data that support the findings of this study are available from the corresponding author upon request.

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Author contributions

T.B. designed the study, contributed to the writing and was involved in recruitment, clinical care and data returns. I.B. and E.R. supervised the genomic analyses of the trial. I.G. and J.A. performed the immunochemistry analyses. M.A., M.C., F.D., F.C., M.-P.S., M.D., C.L., A.G., M.-A.M.R., W.J., B.Y., P.B., N.I., X.T., C.L., J.-C.T., T.L'H., J.-M.F., A.M. and F.D.P. were involved in recruitment, clinical care and data returns. B.V. contributed to writing of the paper. A.T.D. supervised bioinformatics analyses. A.L. and T.F. ran all statistical analyses related to outcomes. M.J. and A.J. are the project managers of the SAFIR02-BREAST trial and centralized the collected samples and data. F.A. designed the study, is the principal investigator of the SAFIR02-BREAST trial, contributed to the writing and was involved in recruitment, clinical care and data returns. All authors approved the final manuscript and contributed to critical revisions of its intellectual content.

Competing interests

F.A. received research funding and served as speaker/advisor (compensated to the hospital) for Roche, AstraZeneca, Daiichi Sankyo, Pfizer, Novartis and Lilly. T.B. received research funding and served as speaker/advisor (compensated to the hospital) for Roche, Novartis, Pfizer, Seattle Genetic, Lilly and AstraZeneca. M.A. received research funding and served as speaker/advisor (compensated to the hospital) for Novartis, AstraZeneca, Seattle Genetics, AbbVie and Pfizer. M.C. received research funding and served as speaker/advisor (compensated to the hospital) for AstraZeneca, Novartis, AbbVie, Sanofi, Lilly, Pfizer, Sandoz, ACCORD, G1 Therapeutic, Pierre Fabre Oncology, Servier and Roche. F.D. received research funding and served as speaker/advisor (compensated to the hospital) for Roche, Novartis, Lilly, Pfizer, Eisai, MSD and AstraZeneca. C.L.-P. received research funding and served as speaker/advisor (compensated to the hospital) for AstraZeneca and Roche. A.G. received research funding and served as speaker/advisor (compensated to the hospital) for AstraZeneca, Pfizer, Novartis, Roche, MSD and Lilly. M.-A.M.R. received research funding and served as speaker/advisor for Pfizer, Novartis, Lilly, Roche, MSD and Myriad. W.J. received research funding and served as speaker/advisor for AstraZeneca, Eisai, Lilly, MSD, Novartis, Pfizer and Roche. B.Y. received research funding and served as speaker/advisor (compensated to the hospital) for AstraZeneca, Roche, Amgen, Novartis, GSK, ECS Progastrin, Pfizer, Merck Serono and Bayer. P.B. served as speaker/advisor for Roche, BMS, IPSEN, Janssen Cilag, Pfizer, Novartis, Astellas and EUSA Pharma. N.I. received research funding and served as speaker/advisor (compensated to the hospital) for Ipsen and Transgene. J.-C.T. received research funding and served as speaker/advisor (compensated to the hospital) for Pfizer and AstraZeneca. J.-M.F. received research funding and served as speaker/advisor for Pfizer and Eisai. J.A. received consultant fees from AstraZeneca, Bayer, BMS, MSD and Roche. The following authors have no disclosures: M.D., A.M., X.T., F.D.P., T.F., I.B., I.G., E.R., A.T.-D., A.L., M.J., A.J., F.C., M.-P.S., B.V., C.L., T.L'H. and F.D.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-020-01189-2.

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Correspondence and requests for materials should be addressed to F.A.

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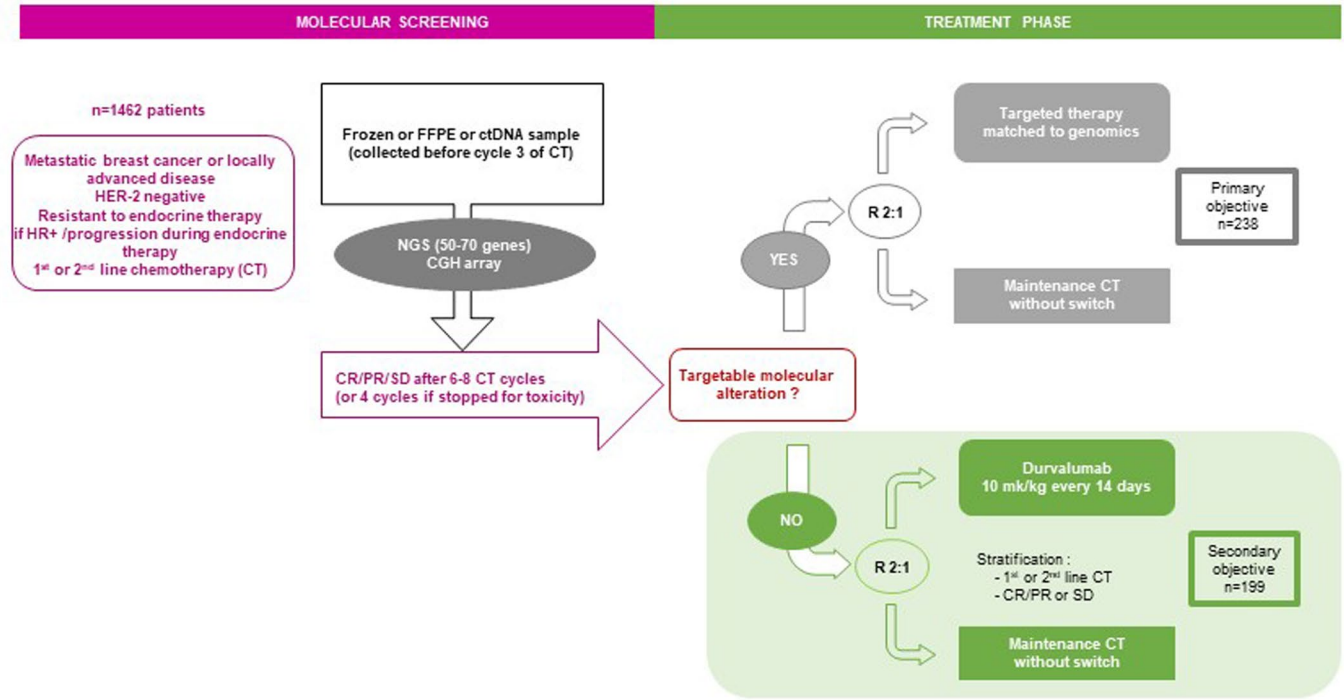
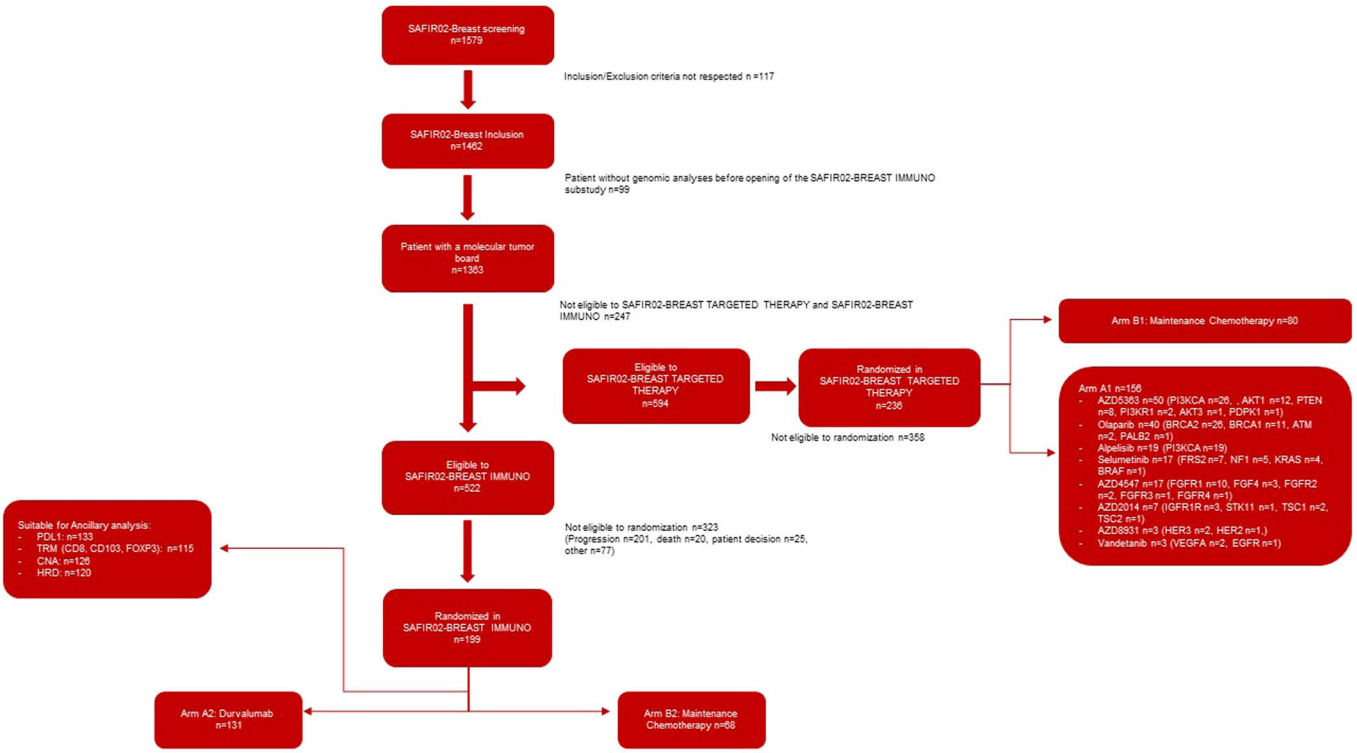
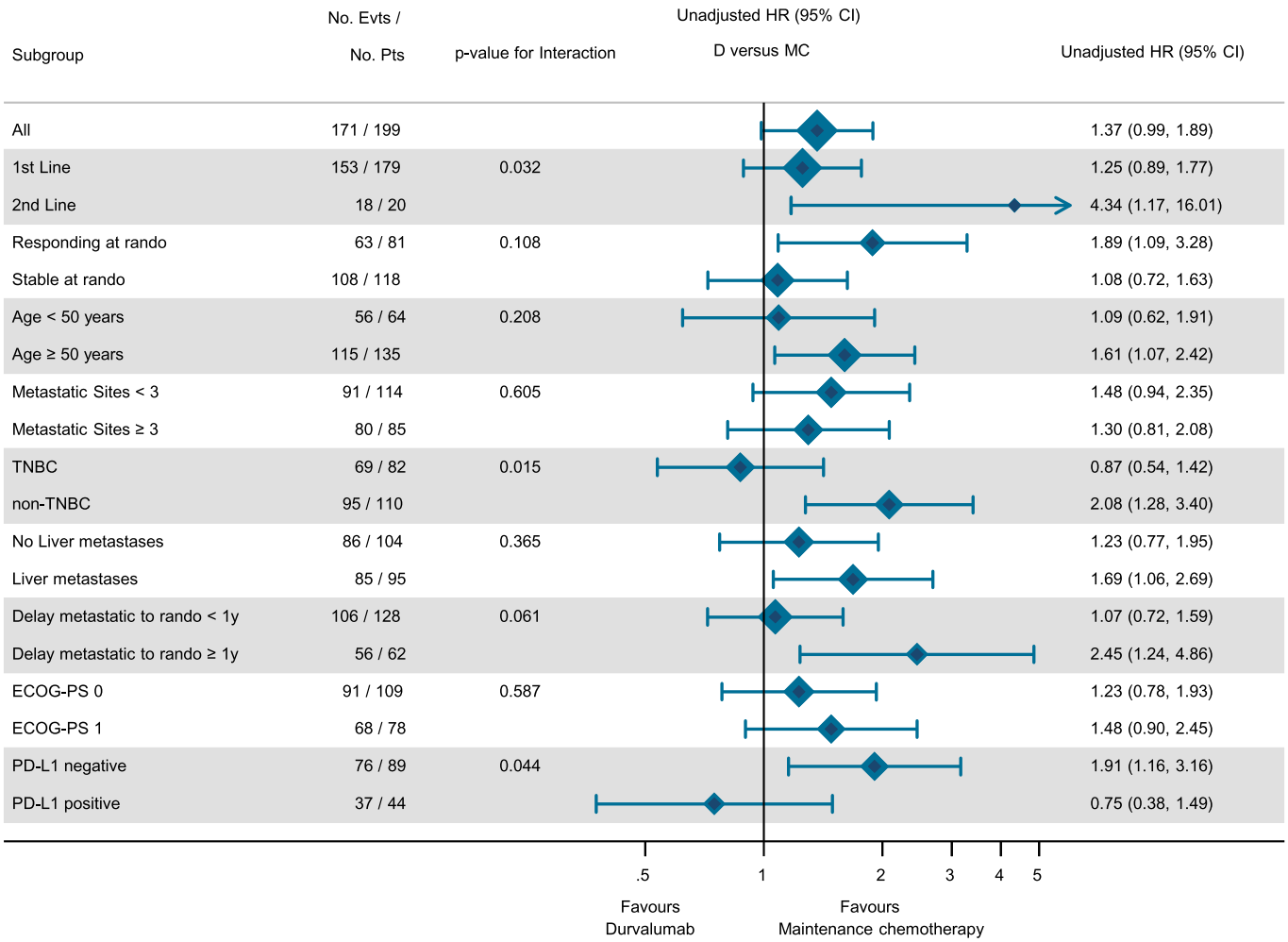


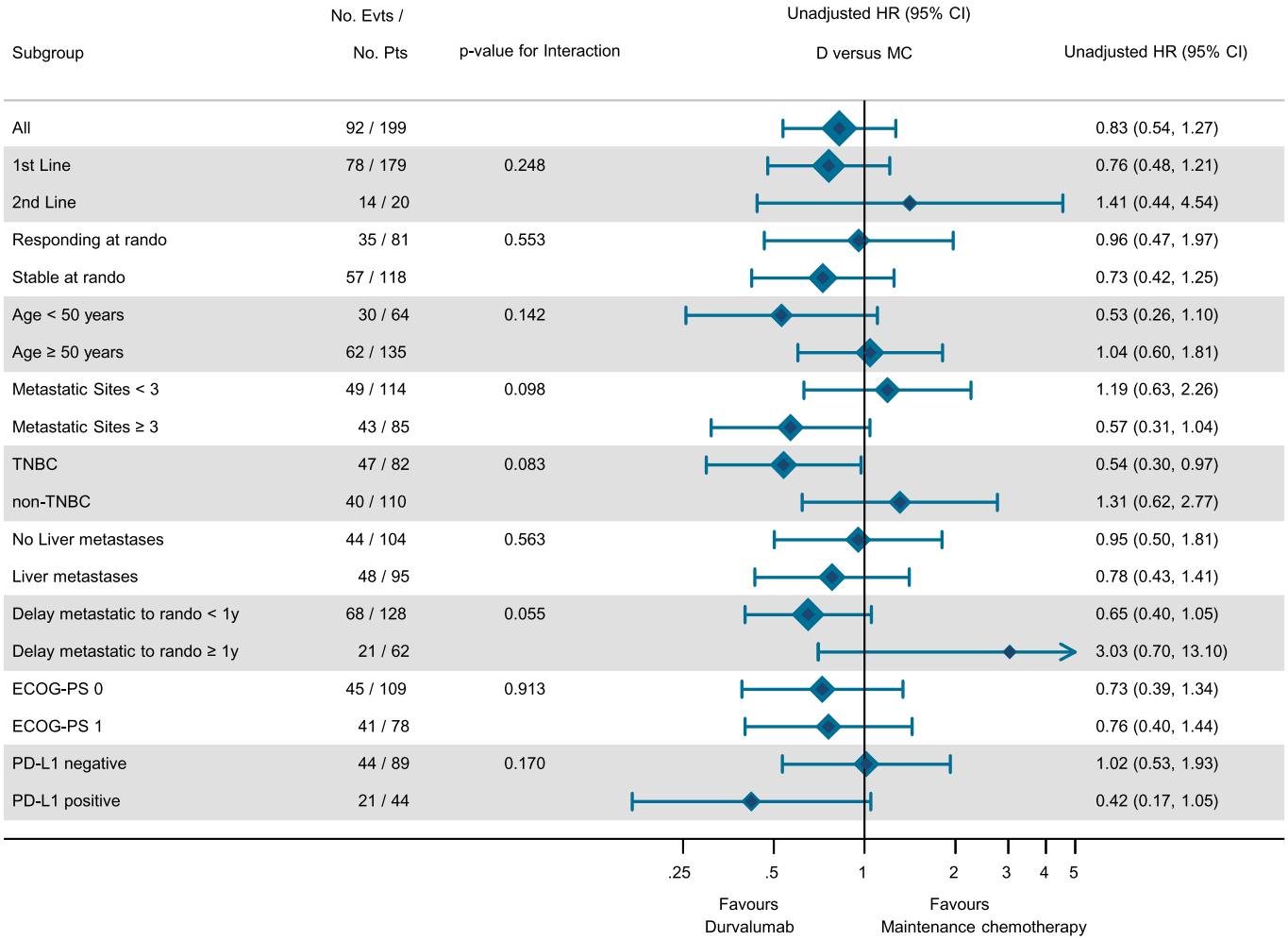
Extended Data Fig. 1 | Study Design. HER**:** Human Epidermal growth factor Receptor; HR: Hormone Receptor; CT: chemotherapy; CGH: comparative genomic hybridization; CNA: copy number alteration; CR: complete remission; PR: partial remision; SD: stable disease; ctDNA: circulating tumor DNA; FFPE: formalin-fixed paraffin-embedded; n: number of patient; R: randomization ratio.



Extended Data Fig. 2 | CONSORT diagram. CNA: copy number alterations; HRD: homologous recombination deficiency; IHC: immunohistochemistry; TRM: tissue-resident memory T cells; n: number of patient.



Extended Data Fig. 3 | PFS in subgroups of interest. The forest plot shows the hazard ratios (diamonds) and 95% two sided confidence intervals (error bars) estimated using an unadjusted Cox proportional hazard model in each subgroup. P-value for interaction between treatment arm and each parameter from a Cox proportional hazard model fitted with the parameter, the treatment arm, and an interaction term between treatment arm and parameter is reported. All statistical tests were two sided. No adjustment was made for multiple comparisons. No. Evts: number of events; No Pts: number of patients; PFS: Progression Free Survival; HR: Hazard Ratio; CI: confidence intervals (two-sided); TNBC: Triple Negative Breast Cancer; ECOG-PS: Eastern Cooperative Oncology Group-Performance Status; D: Durvalumab; MC: maintenance chemotherapy.



Extended Data Fig. 4 | OS in subgroups of interest. The forest plot shows the hazard ratios (diamonds) and 95% two sided confidence intervals (error bars) estimated using an unadjusted Cox proportional hazard model in each subgroup. P-value for interaction between treatment arm and each parameter from a Cox proportional hazard model fitted with the parameter, the treatment arm, and an interaction term between treatment arm and parameter is reported. All statistical tests were two sided. No adjustment was made for multiple comparisons. No. Evts: number of events; No Pts: number of patients; OS: Overall Survival; HR: Hazard Ratio; CI: confidence intervals (two-sided); TNBC: Triple Negative Breast Cancer; ECOG-PS: Eastern Cooperative Oncology Group-Performance Status; D: Durvalumab; MC: maintenance chemotherapy.

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Software and code

Policy information about availability of computer code

Data collection	data collection were performed with CSOnline modul from Ennov Clinical
Data analysis	Copy number variations (CNV) from CytoScan and OncoScan Affymetrix were defined using the R package rCGH (version 1.16.0, under R version 3.6.3). The type of loss -of -heterozygosity (LOH) state were defined using the R package EaCoN available on github (https://github.com/gustaveroussy/EaCoN version 0.3.3). The homologous recombination deficiency (HRD) score was determined using HRDetect. Statistic analysis were performed with Stata software version 16. Vectra 3 microscope and inForm image analysis software (PerkinElmer) were used for immunochemistry analyses. HRP-conjugated amplification systems Discovery UltraMap anti-Rabbit HRP and anti-Mouse HRP (Ventana) were coupled to the fluorophores Opal520 (FITC), Opal570 (Cy3), Opal690 (Cy5), and Opal620 (TexasRed), from the Opal Multiplex Tissue Staining (PerkinElmer) and combined with Vectra 3 microscope and inForm image analysis software version 2.4.6 (PerkinElmer).

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Life sciences study design

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

Sample size	We determined that 166 progression or death would be needed in SAFIR02 Immuno to provide 80%power at a two sided significance level of 0.05 to detect a HR for PFS of 0.63 (increase in median PFS from 3 months to 4.76 months) and a 2:1 randomization. We calculated that the enrollment of 190 patients was necessary to observe the number of required events.
Data exclusions	No data were excluded from analysis
Replication	Regarding the clinical part, replication is not applicable. Regarding the genomic part, experiments were done once. Regarding PD-L1 expression, the SP142 assay was performed following the standardized procedure of the test and evaluated by 2 trained pathologists. Validation of the TRM multiplex panel was performed in non-small cell lung cancer in a previous study: https://www.sciencedirect.com/science/article/pii/S2666379120301695.
Randomization	We used a ratio 2:1 between durvalumab and maintenance chemotherapy. Randomization was stratified using a minimization methods according to the line of chemotherapy (1st line vs 2nd line), the tumor response (Stable disease vs Tumor response)
Blinding	Blinding was not relevant for our study because type of chemotherapy was under the discretion of the investigator

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

Antibodies

Antibodies used	PDL1 staining was assessed using SP142 antibody (Supplier name, catalog number and dilution for SP142 antibody: Ventana, 790-4860, final dilution: 7 micrograms/mL); anti-CD103 (clone EPR4166-2, Abcam, 1:200; Catalog number: rab129202), anti-cytokeratin (clones AE1/AE3, Dako, 1:100, Catalog number: M351501-2), anti-CD8 (clone SP16, Spring Bioscience, 1:100, Catalog Number: M3164) , anti-FoxP3 (clone SP97, Spring Bioscience, 1:150, Catalog Number: M3974).
Validation	Regarding PD-L1, the SP142 assay was performed following the standardized procedure of the test and evaluated by 2 trained pathologists. Validation of the TRM multiplex panel was performed in non-small cell lung cancer in a previous study: https://www.sciencedirect.com/science/article/pii/S2666379120301695

Human research participants

Policy information about studies involving human research participants

Population characteristics	The SAFIR02 program includes patients who present an mBC that does not overexpress Her2 and who had received 0 or 1 line of previous chemotherapy in the metastatic setting. Patients who present an estrogen (ER) and/or progesterone receptor (PR)-positive breast cancer were selected to be resistant to endocrine therapy. In the substudy SAFIR02-BREAST immuno, 199 patients were randomized with median age of 56 years (198 female and 1 male) . 65.8% of patients received neo- or adjuvant chemotherapy for their initial disease. 43% of patient had TNBC and 56% had hormone receptor-positive/

	Her2-negative breast cancer. 106 out of the 108 patients with HR+/Her2- breast cancer received a prior endocrine therapy, including at least one line endocrine therapy in the metastatic setting in 65 patients (60%). 90% patients were randomized after 1st-line induction chemotherapy and 10% after 2d line. 41% of patients presented a tumor response after this induction chemotherapy and 59% a stable disease. 94% of patients received 6 to 8 cycles of induction chemotherapy before the randomization. Patients were selected for not presenting a targetable molecular alteration. Nevertheless, in 14 patients, a somatic genomic alterations was detected in SAFIR0-BREAST trial, but the investigator considered that the proposed targeted therapy was not matched (PIK3CA mutation before alpelisib (n=7), NF1 mutation (n=2), other alteration (n=5)).
Recruitment	First patient was randomized on January 2016 27th and last patient on September 2019 13th. Patients from several centers in France were include in the study. Between 2016 and 2019, we conducted the SAFIR02-BREAST IMMUNO substudy at twenty two study sites in France in accordance with the Declaration of Helsinki, current International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines and all applicable regulatory and ethical requirements. Patients signed informed consent for biopsy, randomization, and use of their biological samples for research purposes.
Ethics oversight	Trial was approved by the French ethics committee, CPP - Ile de France 2, on December 13th 2013 (ref 2013-09-07) and French health authorities, ANSM, on January 14th 2014 ref 2013-001652-36. SAFIR02-BREAST IMMUNO substudy was added to the SAFIR02-BREAST trial in 2015 and was approved by the French ethics committee, CPP - Ile de France 2, on October 15th 2015 (ref 2013-09-07 MS3) and French health authorities, ANSM, on September 15th 2015 (ref 2013-001652-36 MS3).

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
Study protocol	SAFIR02-BREAST Evaluation of the efficacy of high throughput genome analysis as a therapeutic decision tool for patients with metastatic breast cancer EudraCT N° : 2013-001652-36; Sponsor N° : UC-0105/1304
Data collection	Data collection was performed with CSOnline modul from Ennov Clinical. 22 investigational french sites have included patients during the 4 years of recruitment (2016-2019) and have registered data in the eCRF. Risk based monitoring was performed by the promotor of the trial, UNICANCER.
Outcomes	The primary endpoint of the immune substudy 2 is progression-free survival. Secondary endpoint includes Overall Survival. This secondary endpoint was predefined as reported in the clinical protocol. PFS is defined as the time from the date of randomization to the date of the first documented cancer progression (including all invasive breast cancer events), or date of death due to any cause. OS is defined as the time from the date of randomization to the date of death due to any cause.