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Clinical Trial

Rucaparib in patients presenting a metastatic breast cancer with homologous recombination deficiency, without germline *BRCA1/2* mutation



Anne Patsouris ^{a,*}, Kadija Diop ^b, Olivier Tredan ^c, Daniel Nenciu ^d, Anthony Gonçalves ^e, Monica Arnedos ^f, Marie-Paule Sablin ^g, Pascal Jézéquel ^h, Marta Jimenez ⁱ, Nathalie Droin ^j, Ivan Bièche ^k, Céline Callens ^l, Andrea Loehr ^m, Cécile Vicier ^e, Catherine Guerin ⁿ, Thomas Filleron ^o, Fabrice André ^f

^a Department of Medical Oncology, Institut de Cancérologie de l'Ouest-Pays de La Loire, 15 rue Boquel, 49 055 Angers, France

^b Bioinformatic Platform UMS AMMICA Gustave Roussy Campus, 114 Rue Edouard Vaillant, 94805 Villejuif, France

^c Department of Medical Oncology, Centre Léon Bérard, 28 Promenade Léa et Napoléon Bullukian, 69008 Lyon, France

^d Department of Radiology, Institut de Cancérologie de l'Ouest-Pays de La Loire, 15 rue Boquel, 49 055 Angers, France

^e Department of Medical Oncology, Institut Paoli-Calmettes, 232 Boulevard de Sainte-Marguerite, 13009 Marseille, France

^f Department of Medical Oncology, Gustave Roussy, Université Paris Saclay, 114 Rue Edouard Vaillant, 94805 Villejuif, France

^g Department of Medical Oncology, Curie Institute, 26 Rue d'Ulm, 75005 Paris, France

^h Bioinformatics Department, Institut de Cancérologie de l'Ouest-Pays de La Loire, Saint-Herblain, France

ⁱ Unicancer, Paris, France

^j Genomic Core Facility UMS AMMICA Gustave Roussy Campus, Villejuif, France

^k Department of Genetics, Curie Institute and Paris-Descartes University, Paris, France

^l Department of Genetics, Curie Institute and Paris Sciences, Lettres University, Paris, France

^m Translational Medicine Department, Clovis Oncology, San Francisco, USA

ⁿ Department of Biostatistics, Institut de Cancérologie de l'Ouest-Pays de La Loire, Angers, France

^o Department of Biostatistics, Claudius-Regaud Institute, IUCT-oncopole, Toulouse, France

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* Corresponding author. Department of medical oncology, Institut de Cancérologie de l'Ouest-Pays de La Loire, 15 rue Boquel, 49 055 Angers, France.

E-mail address: anne.patsouris@ico.unicancer.fr (A. Patsouris).

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Abstract Background: Breast cancer may present genomic alterations leading to homologous recombination deficiency (HRD). PARP inhibitors have proven their efficacy in patients with HER2-negative (HER2-) metastatic breast cancer (mBC) harbouring germline (g) *BRCA1/2* mutations in 3 phases III trials. The single-arm phase II RUBY trial included 42 patients, 40 of whom received at least one dose of rucaparib. RUBY study assessed the efficacy of rucaparib in HER2-mBC with either high genomic loss of heterozygosity (LOH) score or non-germline *BRCA1/2* mutation.

Patients and methods: The primary objective was the clinical benefit rate (CBR), and the study was powered to see 20% CBR using a 2-stage Simon design.

Results: The primary-end point was not reached with a CBR of 13.5%. Two LOH-high patients, without somatic *BRCA1/2* mutation, presented a complete and durable response (12 and 28.5 months). Whole-genome analysis was performed on 24 samples, including 5 patients who presented a clinical benefit from rucaparib. HRDetect tended to be associated with response to rucaparib, without reaching statistical significance (median HRDetect responders versus non-responders: 0.465 versus 0.040; $p = 0.2135$). Finally, 220 of 711 patients with mBC screened for LOH upstream from RUBY presented a high LOH score associated with a higher likelihood of death (hazard ratio = 1.39; 95% CI: 1.11–1.75; $p = 0.005$).

Conclusion: Our data suggest that a small subset of patients with high LOH scores without germline *BRCA1/2* mutation could derive benefit from PARP inhibitors. However, the RUBY study underlines the need to develop additional biomarkers to identify selectively potential responders. © 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Despite efforts in targeted therapeutic approaches over the past 20 years, metastatic breast cancer (mBC) remains a lethal disease with a median overall survival (OS) of 39 months and the need for further development in personalised medicine [1]. Breast cancer may present genetic alterations in the homologous recombination repair (HRR) pathway, a high-fidelity repair mechanism for double-stranded DNA breaks [2]. Such alterations, including mutations in breast cancer susceptibility genes 1 or 2 (*BRCA1/2*), lead to genomic instability and the development of homologous recombination deficiency (HRD) tumours [3]. Loss of HRR leaves cells needing alternative methods for DNA damage repair, such as base excision repair system, a repair pathway for single-stranded breaks, where the enzyme poly (ADP-Ribose) polymerase (PARP) plays a key role. Dependency to base excision repair may be exploited for therapeutic purposes, PARP inhibition in *BRCA1/2* mutated cancer cells causes unresolved DNA damage leading to cell death [4]. PARP inhibitors efficacy is also due to PARP trapping that results in replication arrest by blocking the replication fork [5].

Based on results demonstrating an improvement of progression-free survival (PFS) from two open-label, multicenter, randomised, phase III trials testing PARP inhibitor versus physician' choice treatment, the two PARP inhibitors olaparib and talazoparib have been approved in previously treated patients with HER2 negative (HER2-) mBC harbouring a germline (g) *BRCA1/2* mutation [6–9]. In these two trials, both triple-negative (TN) and oestrogen receptor positive (ER+)/HER2-mBC patients benefited from PARP

inhibitors. Olaparib effects were further supported in the phase IIIb LUCY study [10]. Veliparib, another PARP inhibitor, also showed its superiority in combination with platinum-based chemotherapy compared with chemotherapy alone in terms of PFS (14.5 versus 12.6 months; $p = 0.002$) in patients with HER2-untreated mBC with *gBRCA1/2* mutation [11]. In adjuvant settings, the OlympiA phase III trial demonstrated a statistically significant improvement of invasive disease-free survival in treated patients versus placebo [12].

If germline or somatic (s) *BRCA1/2* mutations occur in approximately 5% of breast cancer patients [13,14], HRD can also occur due to other mechanisms, without mutation in the *BRCA1/2* genes. These mechanisms, leading to a 'BRCAness profile', encompass somatic and germline mutations, or epigenetic modifications, in other genes that modulate HRR pathway, resulting in genomic instability, reflective of the preferential use of the non-conservative double-stranded DNA breaks repair pathways, such as non-homologous end joining and single-strand annealing [15]. Genomic instability scores such as telomeric allelic imbalance score [16], loss of heterozygosity (LOH) [17], and large-scale state transition (LST) [18] scores have been associated with several BRCAness profile characteristics. HRD profiling has also been approached by mutational signatures, a genomic scar that corresponds to a pattern of mutations strongly associated with *BRCA1/2* mutations [19]. Interestingly, the HRD profile without *gBRCA1/2* mutation also confers sensitivity to PARP inhibitors [5,15]. Recently, Davies *et al.* developed HRDetect, a whole-genome sequencing predictor based on mutational signatures, and other genomic features such as indels with

microhomology at the indel breakpoint junction, indels at polynucleotide-repeat tracts, counts of rearrangements associated with each signature of rearrangements RS1-RS6 and HRD index, to investigate HRD profiles among 560 breast carcinomas. They identified 22% of patients with an HRD profile that could benefit from PARP inhibitors therapy [20]. A phase II trial has shown the activity of talazoparib in somatic or germline HRR mutated genes in patients with HER2-breast cancer. Three out of 12 patients presented a tumour response (2 *gPALB2*, 1 *gCHEK2/gFANCA/sPTEN*) and 3 additional patients (1 *gPALB2*, 1 *sATR*, 1 *sPTEN*) had stable disease ≥ 6 months [21]. Another phase 2 trial, the TBCRC 048 study, has recently shown activity of olaparib in patients that harboured either germline or somatic mutations in HRR pathway genes other than *BRCA1/2* or in addition to *BRCA1/2* somatic mutations. In the somatic cohort, while 8 of 26 patients achieving a partial response under olaparib harboured a *sBRCA1/2* mutation without mutation in other HR-related genes, in the germline cohort, only patients with *gPALB2* mutations (9/27) presented a partial response [22].

Rucaparib, an oral, small-molecule inhibitor of PARP-1, PARP-2, and PARP-3, has demonstrated antitumour activity in various tumour types [23,24]. Rucaparib has been approved by the United States Food and Drug Administration (FDA) and by the European Medicines Agency for the treatment of patients with deleterious BRCA mutation-associated advanced ovarian cancer who have been treated with two or more therapies [25] and recently by the FDA for *BRCA* mutation-associated metastatic castration-resistant prostate cancer [24,26]. Based on data from two open-label, single-arm, multicenter trials, ARIEL2 and Study 10, FDA also approved the companion diagnostic FoundationFocus CDx_{BRCA} LOH, that identifies women with ovarian HRD tumours, for whom treatment with Rucaparib is being considered, by combining analysis of somatic *BRCA1/2* mutation status and the percentage of genome-wide LOH [27–29].

The main goal of the RUBY study was to evaluate the efficacy of rucaparib for the treatment of locally advanced or metastatic HER2-mBC with either high tumour genomic LOH scores or non-germline *BRCA1/2* mutation. A secondary objective was to assess the prognostic value of high genomic LOH scores in HER2-mBC.

2. Material and methods

2.1. Patients and study design

RUBY was a single-arm, open-label, multicentric, phase II study built with a two-stage Simon's design (NCT02505048). All patients provided written informed

consent. Eligible patients were 18 years old and over women with progressive HER2-breast cancer previously treated with at least one line of chemotherapy in the metastatic settings. Patients had a genomic LOH-high score obtained from an available genome-wide human single nucleotide polymorphisms (SNP) array or a *sBRCA1/2* mutation without known *BRCA1/2* deleterious germline mutation. The RUBY study was tightly connected to the SAFIR trials (SAFIR02-Breast; NCT02299999, and SAFIR-TOR; NCT02444390). Genomic LOH score was assessed in patients included in SAFIR trials, and patients with positive BRCAness profiles or with exclusive somatic mutations could participate in the RUBY protocol. Furthermore, patients harbouring *sBRCA1/2* mutations could also come from genomics-driven trials, such as SAFIR02-Breast, MOSCATO (NCT01566019) [30] or PERMED (NCT02342158) trials. Other inclusion criteria comprised measurable disease (according to response evaluation criteria in solid tumours version 1.1 [RECIST v1.1]), an Eastern cooperative oncology group performance status 0 or 1, and a 21-day washout period from last chemotherapy or targeted therapy with resolution of all toxicities to grade ≤ 1 , excluding alopecia. Main exclusion criteria included known *gBRCA1/2* deleterious mutation, contraindication to rucaparib treatment, previous treatment with a PARP inhibitor, less than 14 days from radiotherapy, spinal cord compression and/or symptomatic or progressive brain metastases, problem with intestinal absorption, severe or uncontrolled systemic disease, history of myelodysplastic syndrome, and haematopoietic function or organ impairment. The national ethics committee approved the study.

2.2. Procedure and assessments

Patients were treated with 600 mg oral rucaparib twice a day until disease progression, unacceptable toxicity, intercurrent conditions that preclude continuation of treatment, or patient refusal. Toxicity management and dose reduction followed a summary of products characteristics recommendations and local standard practice. Clinical and laboratory examinations were performed every 4 weeks after treatment initiation. The safety was assessed and graded by National Cancer Institute — common terminology criteria for adverse events version 4.03 (NCI-CTCAE v4.03) every 4 weeks from treatment initiation until the end of treatment. Assessment of response to treatment for the therapeutic decision was based on investigator-reported measurements on target and non-target lesions and carried out according to RECIST v1.1 with computed tomography scans or magnetic resonance imaging repeated every 8 weeks. A central review was set up to confirm investigator-reported image measurements for all patients enrolled during the first stage of the study and patients with a response to treatment in the second stage.

2.3. Genomic LOH assessment

As previously indicated, RUBY and SAFIR trials, especially SAFIR02-Breast and SAFIR-TOR trials were tightly connected. All alive, non-germline *BRCA1/2*-mutated patients with a CytoScan HD or OncoScan copy number variation (CNV) profile prospectively generated from freshly collected metastatic tumour samples for SAFIR02-Breast or SAFIR-TOR studies were screened for HRD. Tumour biopsies were performed on purpose for studies before initiation of a first line of chemotherapy in the metastatic setting, in HER2-patients, for SAFIR02-Breast, and before initiating treatment with exemestane plus everolimus as per marketed indication before any chemotherapy, in ER+/HER2-patients for SAFIR-TOR. A local pathologist assessed biopsies from metastatic lesions to retain samples with more than 30% of cancer cells. Methods for DNA extraction have been previously reported [31] DNA CNVs microarrays analyses were performed using Affymetrix technology (Thermo Fisher Scientific company). OncoScan™ CNV Assay Kit was used for FFPE tissue samples (designed for degraded DNA), and the CytoScan™ HD Array Kit was used for the fresh-frozen tissues.

Affymetrix raw files (.cel) from SAFIR02-Breast/SAFIR-TOR underwent a second pseudonymisation procedure consisting of changing the patient ID code. A hexadecimal editor software (Frhed v1.6.0, <http://frhed.sourceforge.net>) was used to search and replace the patient ID code. To exclude any injury in the structure of the file after this procedure, each recorded file was tested to validate its integrity.

Pseudonymised array data files were then sent to Clovis Oncology for HRD assessment, and the percentage of genome-wide LOH was calculated using the method previously described [28]. For each sample from SAFIR02-Breast and SAFIR-TOR studies, LOH regions were inferred across the 22 autosomal chromosomes of the genome using the computed minor allele frequencies of the SNPs sequenced in the Affymetrix assays. LOH inference was based on Biodiscovery's implementation of the published ASCAT (allele-specific copy number analysis of tumours) methodology [32,33]. LOH regions spanning across $\geq 90\%$ of a whole chromosome or chromosome arm were excluded from the calculation because these LOH events are likely because of non-HRD mechanisms [34].

Hence, for each tumour, the percentage of the genome with LOH was computed as 100 times, the total length of non-excluded LOH regions divided by the total length of the consultable genome.

In equation form:

$$\% \text{ genome with LOH} = 100 * \frac{\sum (\text{lengths of non-excluded LOH regions})}{\sum (\text{lengths of genome with SNP coverage}) - \sum (\text{lengths of excluded LOH regions})}$$

We prespecified a cutoff of 18% or more to define a 'high' genomic LOH score for breast carcinoma. This score was estimated to capture the top 25% of LOH scores based on previous analysis of The Cancer Genome Atlas (TCGA) [2] microarray (<https://www.cancer.gov/tcga>), and SAFIR01 [35] (NCT01414933) plus SAFIR02-Breast microarray datasets (n = 675).

2.4. Outcomes

To assess the efficacy of the rucaparib treatment, the co-primary efficacy end-points were the clinical benefit rate (CBR), and if significant, the overall response rate (ORR) according to a hierarchical procedure. The CBR was defined as the proportion of patients whose best overall response was either a complete response, a partial response, or a stable disease lasting at least 16 weeks. The ORR was defined as the proportion of patients whose best overall response was either complete or partial response. The secondary end-points were PFS, OS, and safety. PFS was defined as the time from the first dose of rucaparib to disease progression or death from any cause. OS was defined as the time between the date of inclusion and death from any cause. The safety profile of rucaparib was characterised by the occurrence of adverse events during the on-treatment period (defined as the period from the time of the first dose of study medications up to 30 days after the last dose).

2.5. Statistical analysis

Under the assumption of a minimum CBR of 20%, we calculated that we needed to include 37 patients to achieve a power of 90% at a two-sided α level of 10%. 41 patients were required considering attrition of 10% of non-evaluable patients. A success rate $>20\%$ was considered to have clinical relevance, and a success rate of $>40\%$ was considered to have a high clinical interest.

We applied a two-stage Simon's design to stop the trial early for futility in case of the low proportion of patients with clinical response. The first stage required 19 patients. We planned to close the study if less than 4 patients achieved a clinical benefit to rucaparib treatment as confirmed by the central review of computed tomography scans. Otherwise, we planned to continue the study until 41 patients were included. We required at least 11 of 37 evaluable patients to achieve a clinical response to claim the success of the study.

We analysed the main criteria in the population, including patients who received at least one dose of treatment and had a first, post-baseline, tumour assessment according to RECIST v1.1 (per-protocol population). A patient who discontinued rucaparib before the first RECIST tumour assessment for toxicity or clinical progression were kept in the efficacy population (modified intent-to-treat [mITT] population) and considered as failures (supplementary Figure A.1).

CBR and ORR were reported by percentage with their 95% confidence interval (CI) calculated using the binomial Clopper-Pearson method. The secondary endpoints PFS and OS were estimated using Kaplan–Meier methods on the mITT population. At the time of analysis of PFS or OS, data were censored at the date of the last follow-up visit for patients without clinical disease progression assessment and alive. Patients who received any amount of study treatment were included in the safety analyses. The frequency and percentage of events were summarised according to the primary system organ classes (SOC) and by the preferred term of the medical dictionary for regulatory activities (MedDRA, version 19.1). Specific attention was provided to the grade >2 events. Data analysis were performed using R (version 3.6.0) software.

2.6. Prognostic value of genomic LOH score

To identify patients for the RUBY study, we analysed microarray data from the SAFIR02-Breast and SAFIR-TOR studies. 711 patients (n = 620 from SAFIR02-Breast, n = 91 from SAFIR-TOR studies) were successfully tested for a genomic LOH score. The proportion of LOH-high score was compared between early breast cancer from TCGA dataset (n = 1004) and mBC from SAFIR02 trial (n = 620), using chi-square or Fisher exact test. The prognostic value of high genomic LOH was assessed only on patients from the SAFIR02-Breast trial for which outcome data were available. OS was defined in SAFIR02-Breast as the time from inclusion to death from any cause and was estimated using the Kaplan–Meier method with 95% CI. Patients alive at the time of analysis were censored at their last follow-up date. Univariable and multivariable analyses were performed using the log-rank test and Cox proportional hazards model, respectively. Significant factors (i.e. $p \leq 0.05$) in the univariable analysis were included in the multivariable analysis. The prognostic value of genomic LOH score analysis was performed using Stata Version 16.

2.7. BRCA1 and BRCA2 deficiency prediction with HRDetect

As an exploratory study, a whole-genome sequencing (WGS) was performed as previously described in a sub-population of RUBY patients [14,36].

The complete methods for BRCA1 and BRCA2 deficiency prediction with HRDetect are detailed in supplementary data (Appendix B). Briefly, for data pre-processing, whole-genome sequence reads were mapped to the human genome build hg19 using Burrows-Wheeler Aligner MEM algorithm (BWA v0.7.17) compatible with Genome Analysis Toolkit (GATK). The workflow described in Genome Analysis Toolkit Best Practices for somatic short variant discovery and germline short variant discovery was followed for

Table 1
Baseline demographic and disease characteristics.

Characteristic	Patients, N (N = 40)	(%)
Age, median (range), years	54 (27–76)	
Phenotype (metastatic biopsy)		
ER+ and/or PR+	21/40	52.5
TNBC	19/40	47.5
Genomic instability		
High genomic LOH	35/40	87.5
<i>sBRCA1/2</i> mutation	5/40	12.5
Metastases		
De novo	6/39	15.4
Number of metastatic site		
<3	15/39	38.5
≥3	24/39	61.5
Visceral involvement		
Liver and/or lung metastases	33/39	84.6
Liver metastases	29/39	74.4
ECOG Performance Status		
0	23/40	57.5
1	17/40	42.5
Median of previous lines of chemotherapy in metastatic settings	2 (1–6)	
Number of patients receiving platinum salts	10/40	25

ECOG: Eastern Cooperative Oncology Group, ER: oestrogen receptor, LOH: loss of heterozygosity.

PR: Progesterone receptor, TNBC: Triple-negative breast cancer.

somatic and germline variant calling, respectively. ANNOVAR program (v20191107) was used to annotate variants. The bioconda package *cnv_facets* (v0.15.0, https://github.com/dariober/cnv_facets/) was used to detect allele-specific CNVs in tumour samples compared to a matched normal sample. We used a tool called Manta (v1.6.0) to detect somatic structural variants with defaults parameters. The HRDetect-pipeline was downloaded from the Github repository: <https://github.com/eyzhao/hrdetect-pipeline> and modified to be used for the RUBY study. The somatic short variants, CNVs and structural rearrangements were

Table 2
Disease characteristics of RUBY patients responding to rucaparib treatment (central review).

Patient number	Inclusion criteria	Phenotype	Best response/ Duration of treatment
1	High LOH score	ER+/ PR+	Complete response/ 28.5 months
2	High LOH score	TNBC	Complete response/12 months
5	<i>sBRCA2</i> mutation (+g <i>PALB2</i> mutation)	ER-/PR+	Partial response/6.5 months
4	High LOH score	ER+/-PR-	Partial response/7.2 months
7	High LOH score	TNBC	Partial response/4.5 months
3	<i>sBRCA1</i> mutation	ER+/ PR+	Stable disease/7.3 months

ER: oestrogen receptor, LOH: loss of heterozygosity, PR: progesterone receptor, TNBC: triple-negative breast cancer, s: somatic, g: germline.

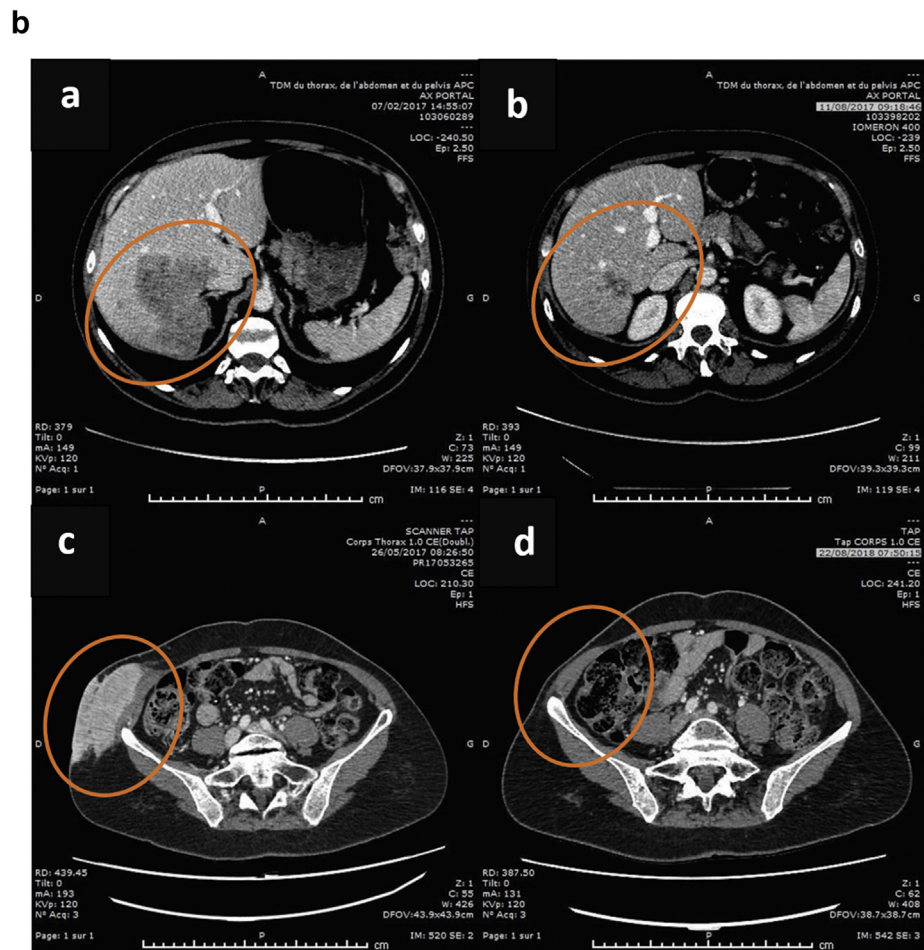
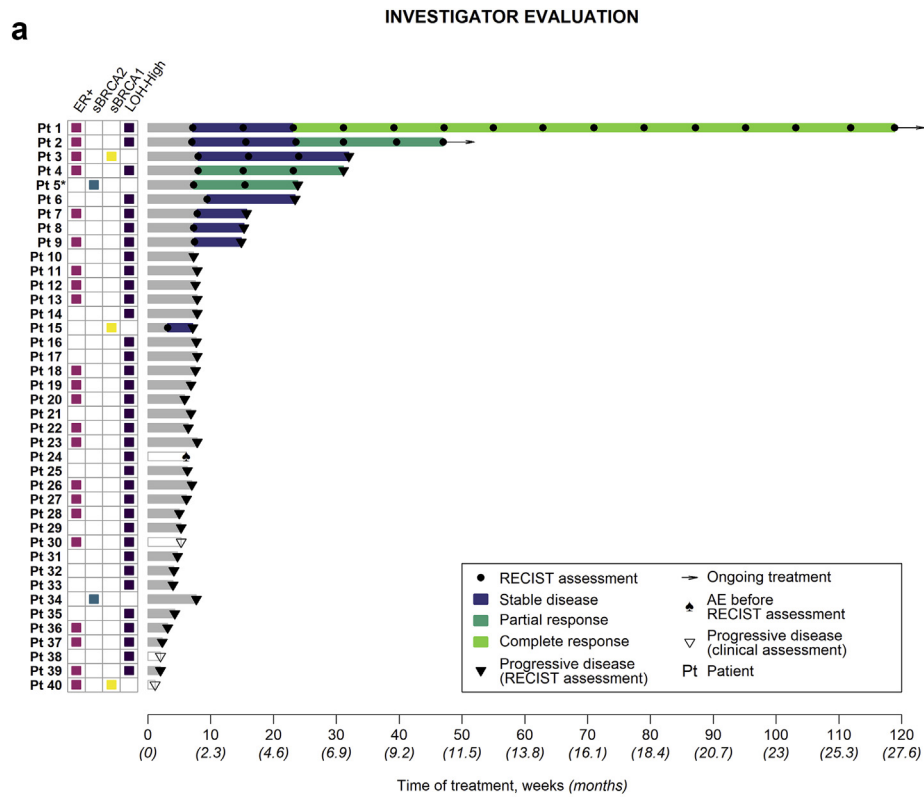


Fig. 1. Investigator response evaluation in RUBY. a. Swim lane plot of duration of response in RUBY patients according to investigator evaluation. Each bar represents an individual patient with the length corresponding to the time on study drug. White bars represent

used as input of the HRDetect pipeline. The HRDetect scores were then determined as previously described [20].

3. Results

3.1. Efficacy

Between January 9, 2016, and December 15, 2018, 12 centres enrolled 42 patients in the RUBY study. Two patients were excluded before treatment, one for lack of measurable target lesion at inclusion (according to RECIST v1.1) and one for disease progression (brain metastasis) before treatment initiation, and 40 patients received at least one dose of rucaparib (Supplementary Fig. 1).

The median age at inclusion was 54 years (range: 27–76). Thirty-five patients (87.5%) were included based on a high genomic LOH score and 5 patients (12.5%) based on a *sBRCA1/2* mutation. Details of the *sBRCA1/2* mutations are provided in supplementary table B.1. Tissues were obtained from liver biopsies in 18 (51%) patients. 19 patients (47.5%) had triple-negative breast cancer (TNBC), assessed on metastatic samples. Two patients, with an ER+/HER2-phenotype on their primary tumour, presented a TNBC phenotype on their metastatic samples. 33 patients (84.6%) presented visceral metastases, including 29 (74.4%) with liver metastasis. Ten patients received prior platinum salt treatment, including 2 patients (5%) in the adjuvant setting. Patients characteristics are summarised in Table 1.

The median duration of follow-up was 25.8 months (95%CI: 14.7–not reached). At the time of data cut-off (January 8, 2020), 37 patients (92.5%) had stopped their treatment because of disease progression and 1 (2.5%) due to toxicity. Two patients (5.0%) were still under treatment, and 29 (72.5%) had died. Of the 40 patients who received at least one dose of rucaparib, 4 patients left the trial before the first RECIST evaluation, including 3 patients because of clinical disease progression and one patient due to toxicity (all 4 are considered as treatment failures in the mITT population).

At the end of the first step of the 2-stage Simon study, the blinded central review confirmed clinical benefit for 4 out of the 17 evaluable patients, allowing to proceed with step 2. At the final analysis, the investigators evaluated 37 patients, 5 of which showed a clinical benefit per investigator assessment, including 1 complete response, 3 partial responses and 1 stable disease ≥ 16 weeks. The central review confirmed 2 complete responses, 3 partial responses and 1 stable disease ≥ 16 weeks. Responders' characteristics are summarised in Table 2. One patient (#6) showed stable

disease with a duration of 15.7 weeks, confirmed by central review, thus not included in the CBR events (< 16 weeks). Investigator-assessed CBR was 13.5% (5/37 patients; 95% CI: 4.5–28.8). For two patients (#1, #4), rucaparib treatment produced an outlier tumour shrinkage. In addition, 1 patient harbouring *sBRCA1* mutation (#3) showed stable disease, and 1 patient with a *sBRCA2* mutation and a *gPALB2* mutation (#5) showed a partial response (Fig. 1). The median duration of rucaparib treatment was 47 days (range: 8–668 days). Median PFS was 1.7 months (95% CI: 1.4–1.8), and median OS was 6.7 months (95% CI: 5.6–12.5). Among the 8 patients who had previously received platinum salts in the metastatic settings, 5 presented a partial response to this previous therapeutic. Only 1 patient previously treated by platinum salts benefited from rucaparib, but the small number of patients did not allow statistical analysis.

3.2. Safety

Of the 40 patients who received at least one dose of rucaparib (safety population), 12 patients (30%) had at least one dose reduction and 12 (30%) a treatment interruption. One patient discontinued rucaparib because of toxicity consecutive to grade 3 anaemia and thrombocytopenia before the first tumour assessment. Two other patients who discontinued treatment mainly due to disease progression were concomitantly presenting a grade 3 anaemia and lymphopenia and a grade 3 neutropenia and grade 4 thrombocytopenia, respectively.

The most frequently reported adverse event (AE) ($\geq 20\%$ of the safety population) were asthenia (71%), aspartate aminotransferase increase (66%), gamma-glutamyltransferase (GGT) increase (58%), anaemia (48%), alanine aminotransferase increase (40%), lymphopenia (38%), blood alkaline phosphatase increase (34%), nausea (36%), thrombocytopenia (20%) and hypercholesterolaemia (20%). Grade 3 AE were mostly related to liver enzyme disorders with aspartate aminotransferase (25%), GGT (20%), and alanine aminotransferase (15%) increase. Grade 3 asthenia was reported by 18% of the patients. The most frequently reported grade 4 AE were GGT increase (8%), anaemia and thrombocytopenia (5%), and blood alkaline phosphatase increase and hypertriglyceridemia (3%). Of note, 22 patients (55%) had some degree of hepatic cytolysis at baseline. Twelve patients (30%) had at least 1 serious AE, predominantly blood and lymphatic system disorders (5 patients) (supplementary table B.2).

patients that progressed or presented adverse event before the first RECIST assessment *Patient 5 harbours a germline PALB2 mutation. b. CT-scan of 2 RUBY patients responding to rucaparib treatment. Portal venous phase of a right lobe liver metastasis for patient #4 at baseline evaluation (a) and after 6 months of treatment (partial response) (b). Portal venous phase of muscle and skin metastasis located near the right iliac wing for patient #1 at baseline evaluation (c) and after 15 months of treatment (complete response) (d). CT, computed tomography.

Table 3
Genomic loss of heterozygosity score in metastatic (SAFIR02-breast) and early (TCGA dataset) breast cancer.

Genomic LOH score	TCGA BRCA (n = 1004)	SAFIR 02-breast (n = 620)	
Overall population (n = 1624)			
Low	800 (79.7%)	414 (66.8%)	p < 0.0001
High	204 (20.3%)	206 (33.2%)	
ER + /HER2- (n = 815)			
Low	393 (86.2%)	252 (70.2%)	p < 0.0001
High	63 (13.8%)	107 (29.8%)	
TNBC (n = 356)			
Low	47 (41.6%)	147 (60.5%)	p = 0.0009
High	66 (58.4%)	96 (39.5%)	

ER: oestrogen receptor; LOH: loss of heterozygosity, PR: progesterone receptor; TNBC: triple-negative breast cancer.

3.3. Prognostic value of high genomic loh score

Six hundred and twenty mBC patients from SAFIR02-Breast and 91 from SAFIR-TOR studies were successfully assessed for a genomic LOH score. All patients presented with mBC, and genomic LOH score was determined on tumour material obtained when patients developed metastases. ‘High’ genomic LOH was defined as having genomic LOH across 18% or more of the genome. 220 (30.9%) presented a high genomic LOH score. We only used SAFIR02-Breast data to determine the prognostic value of the LOH score. Among the 620 SAFIR02-Breast LOH profiles, 206 (33.2%) presented a high genomic LOH score. Patients’ characteristics are summarised in [supplementary table B.3](#). This proportion was lower in the overall population of the early stage TCGA BRCA dataset: 204/1004 (20.3%). In ER+/HER2-, mBC was associated with a higher rate of high LOH score (29.8%) compared to early breast cancer from TCGA (13.8%). In opposite, metastatic TNBC was associated with a lower rate of high LOH score (39.5%) compared to early TNBC (58.4%) ([Table 3](#)). In the metastatic settings, a high genomic LOH score was associated with a higher rate of *sBRCA1/2* mutation compared to a low LOH score (2.9% versus 0.5% p = 0.0187) ([supplementary table B.4](#)). In a multivariate analysis, high LOH score was associated with worse OS in the overall SAFIR02-breast population (hazard ratio = 1.39; 95% confidence interval [CI]: 1.11–1.75; p = 0.005) and in the subgroup of ER+/HER2-patients (adjusted hazard ratio = 1.45; 95% CI, 1.06–1.99; p = 0.019). In the metastatic TNBC population, a similar trend for a worse prognosis in patients with high LOH score than patients with low LOH scores, despite the lack of statistical significance (adjusted hazard ratio = 1.32; 95% CI; 0.95–1.84; p = 0.095) ([Fig. 2](#) and [Supplementary tables B.5 to B.7](#)). In a univariate analysis, the site of metastatic biopsy (liver or not) did not affect the prognostic value of a high LOH score.

3.4. HRDetect

Of the 40 RUBY patients treated with rucaparib, 24 had tumour DNA available paired to constitutional DNA for whole-genome analysis. The average depth for tumour sequencing was 23.19X. This analysis included 14 patients with ER+/HER2-phenotype and 10 patients with TNBC phenotype. Among them, 2 patients harboured a *sBRCA1* mutation and 1 patient with a *sBRCA2* mutation. Five of the 6 patients who presented a clinical benefit from rucaparib, according to central review, were also included in this dataset.

Of the five responders, two patients were identified as having a probability of *BRCA1/2* deficiency per HRDetect exceeding 0.7: patient #1 (LOH score 19%; HRDetect score: 0.72154) presented a complete response and was still under treatment (>28.5 months), and patient #7 (LOH score 39%; HRDetect score: 0.93522) presented a partial response. None of them was included on the basis of *sBRCA1/2* mutation. Two other responding patients harboured a probability of 0.465 (patient #4) and 0.024 (patient #2) and presented a liver partial response and complete response, respectively. Finally, the fifth responding patient (patient #3) had a stable disease >16 weeks and was included based on *sBRCA1* mutation (c.171del (p.pro58leufs*11)) harboured a low probability at 1.062e-4. HRDetect score tended to be higher in patients who presented a benefit from rucaparib versus patients without CBR, although not significant. The mean HRDetect score in responders versus non-responders was 0.465 versus 0.040 (p = 0.2135). The probability of *BRCA1/2* deficiency exceeding 0.7 was significantly correlated with tumour response to rucaparib (p = 0.0362) ([Fig. 3](#)). Patient #34 with *BRCA2* truncated mutation (c.1135G > T, p.(Gly379Ter)) and patient #40 with *BRCA1* truncated mutation (c.1762_1768del, p.(Ser588Valfs*2)) who did not retrieve any clinical benefit from rucaparib treatment presented a low HRD probability of 0.002 and 0.012, respectively.

4. Discussion

The RUBY trial did not meet its primary end-point with a CBR of 13.5%. However, our data support the potential benefit of a PARP inhibitor with prolonged responses in a subset of patients with mBC beyond *gBRCA1/2* mutation. Indeed, results from the RUBY trial showed the efficacy of rucaparib as a monotherapy treatment in 6 patients with either *sBRCA1/2* mutation or high genomic LOH, as assessed by central review, including an exceptional liver partial response and 2 complete responses with a 12- and 28.5-months treatment duration in patients enrolled based on high LOH score.

Moreover, the RUBY trial shows some limitations related to the small sample size (40 patients) and the single-arm design. In the absence of randomisation, we cannot evaluate whether rucaparib could be more efficient than

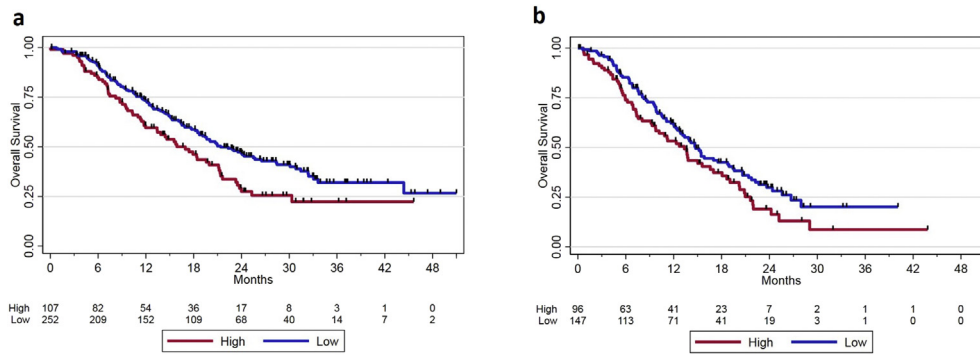


Fig. 2. Overall survival based on high versus low LOH score in SAFIR02-breast. (a) ER+/HER2-breast cancer, adjusted HR = 1.45 (95% CI: 1.06–1.99), $p = 0.019$; Cox multivariate analysis adjusted on previous chemotherapy, liver metastasis (Yes versus No), number of the metastatic site (<3 versus ≥ 3), and grade SBR (1/2 versus 3). (b) Triple-negative metastatic breast cancer. Adjusted HR = 1.323 (95% CI: 0.953–1.839), $p = 0.095$; Cox multivariate analysis adjusted on age at inclusion (≤ 50 versus > 50), liver metastasis (Yes versus No), and number of metastatic site (<3 vs ≥ 3). LOH, loss of heterozygosity.

usual chemotherapy in patients with high genomic instability associated with poor outcomes. Finally, most of the patients are highly pre-treated with a median of two previous lines of chemotherapy, which does not include endocrine therapy for ER+/HER2-mBC. This could contribute to explain, in part, the poor outcomes observed in a population selected on the basis of high genomic instability, as discussed in the following. This clinical trial questions the best predictor of PARP inhibitor efficacy.

4.1. Are the genomic scars captured by the genomic scores, related to HRD or do they reflect of genome evolution and instability?

The poor study outcomes could be explained by patient selection. Indeed, the LOH score was used to select patients as a surrogate marker of HRD [37]. LOH scores

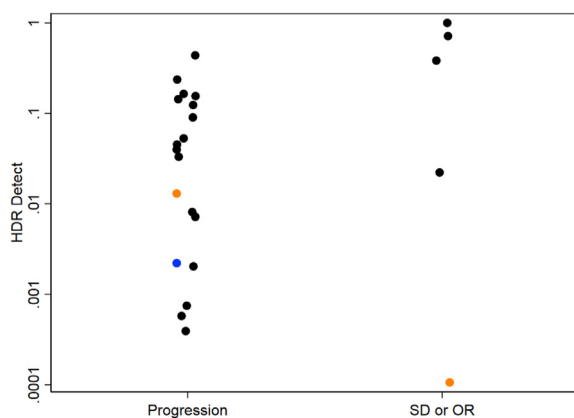


Fig. 3. Correlation of HRDetect score and tumour response to rucaparib in RUBY. On the 24 samples assessed for whole-genome analysis, the probability of *BRCA1/2* deficiency exceeding 0.7 was significantly correlated to tumour response to rucaparib ($p = 0.0362$). Black points represent patients included on the basis of high LOH score, orange points patients harbouring somatic *BRCA1* mutation and the blue point patient harbouring a somatic *BRCA2* mutation. HRD, h.

have been developed in ovarian cancer and primary breast cancers [28] but it is not clear whether they apply to heavily pretreated mBC. As previously reported, the genome evolves when becoming drug-resistant and during the metastatic process [14,38]. Genome evolution is correlated with an increase in the percentage of genomic scars previously associated with HRD. Whether HRD creates these genomic scars, or whether it is a marker of genome evolution or instability unrelated to HRD remains unclear. Here, we showed that high genomic LOH was more frequent in ER+/HER2-mBC than TCGA ER+/HER2-early breast cancer samples. The opposite was observed in TNBC, which could suggest that in this phenotype, tumour cells could not survive a high degree of genomic instability with a selection of the tumour clones escaping adjuvant systemic treatment [37]. Furthermore, the genomic LOH score does not allow an accurate prediction of HRD genomic scars clonality; therapeutic resistance can thus be associated with the selective advantage of clonal selection during the course of the disease [5]. On the other hand, genomic scars reflect the tumour's history rather than its current HRR status which may re-acquire, through various mechanisms of HRR proficiency, such as reversion mutation, especially in patients previously pretreated with DNA crosslinking agents [39].

4.2. PARP inhibitors efficacy beyond *gBRCA1/2* mutations

Pathogenic genomic alterations affecting HRR-related genes are associated with genomic features of HRD, especially in the case of bi-allelic alterations [40]. Most of the current trials testing PARP inhibitors in non-germline *BRCA1/2*-mutated advanced breast cancer, such as the phase II studies VIOLETTE (NCT03330847), NOBROLA (NCT03367689), and DOLAF (NCT04053322), are using a targeted next-generation sequencing panel of HRR genes considering the same contribution of all HRR genes to the

HRD profile. Among patients without *gBRCA1/2* mutations, most of those benefiting from PARP inhibitors harboured *gPALB2* mutations and, to a lesser extent, *sBRCA1/2* mutations. In addition, the TBCRC 048 trial recently showed activity of olaparib in either *gPALB2* mutation or *sBRCA1/2* mutations but not with *ATM* or *CHEK2* mutations [41]. In another phase II study, 3 out of 6 patients who benefited from talazoparib presented a *gPALB2* mutation [21]. Recently, the PETREMAC phase 2 trial has evaluated the efficacy of olaparib as monotherapy in 32 patients with early TNBC. Excluding 5 germline mutations (1 *gPALB2* and 4 *gBRCA1/2*), 6 out of 14 responders harboured somatic mutations in HRR genes (*BRCA1*, *ATR*X, components of histone methyltransferase complex (*SETD2*, *MEN1*), *PTEN*, *EMSY*) and 6 responders carried *BRCA1* methylation without mutations [42]. Interestingly, mutations in other DNA damage repair pathway genes, such as *TP53*, were observed, associated with HRR genes mutations, questioning their involvement in PARP inhibitor response. In RUBY, 2 of 5 patients that harboured *sBRCA1/2* mutation benefited from rucaparib with stable disease (*BRCA1* mutation) and a partial response (*BRCA2* mutation). This last patient also presented a *gPALB2* mutation. Of note, the two patients with prolonged complete response were selected based on a high genomic LOH score. However, genomic LOH analysis alone seems to be insufficient to completely predict HRD status [17]. In phase III, ARIEL 3 trial, 30% of patients with BRCA wild-type and low-LOH genomic score ovarian carcinoma experienced clinical benefit for more than a year in the rucaparib group versus 5% in the placebo group [43]. Similar benefits of rucaparib treatment were reported in ARIEL 2 trial [28]. The question remains whether genomic LOH score is the best biomarker to select patients with mBC that could benefit from PARP inhibitors. Other predictors have been tailored, included HRDetect.

4.3. Clinical validity of HRDetect

Exposure to different mechanisms of DNA damage and repair lead to specific patterns of mutations that constitute mutation signatures, with more than one process operative in most cancers [19]. The base-substitution signature 3 has previously been shown to distinguish *gBRCA1/2* deficient and more recently *gPALB2* mutation (nonsense and frameshift missense) from sporadic cancer in a subset of breast cancer patients but is not discriminating enough when used alone [44]. Indeed, at least five signatures have been observed resulting from a *BRCA1/2* gene defect, including microhomology-mediated indels and more deletions especially those observed in *BRCA1/2* mutant breast cancers, reflect the preferential use of error-prone mechanisms of DSB repair, such as non-homologous end-joining process [45–47]. The whole genome-based

predictor, HRDetect, developed by Davies *et al.*, is based on a lasso logistic regression model applied on HRD copy number indices, twelve base-substitution, 2 indel and 6 mutational signatures previously extracted [20]. HRDetect was investigated on 560 breast carcinoma samples and was very performant to identify patients with HRD profile (up to 22%) with high sensitivity even in the case of low-coverage genome sequencing using an absolute probability cutoff of 0.7²⁰. Most notably, HRDetect was more performant in identifying *BRCA1/2* defective tumours than the combination of LOH, LST, and telomeric allelic imbalance score forming the HRD-score (score >42 in HRD tumours) [20,46]. In the SCAN-B project, HRDetect brings prognostic value with better outcomes on adjuvant chemotherapy in 59% of 254 early TNBC predicted as HRDetect-high, whatever the epigenetic/genomic underlying mechanism, versus low (invasive disease-free survival HR = 0.42, 95% CI = 0.2–0.87) [48]. In the phase II window trial RIO (n = 43), HRDetect score >0.7 identified 69% (18/26) of untreated primary TNBC that harboured HRD (HRDetect+), including identification of all tumours with genomic or epigenetic alteration in HRR pathways (somatic mutation of *BRCA1*, *BRCA2*, and *PALB2*, and promoter methylation of *BRCA1* and *RAD51C*) [49]. HRDetect + tumours were associated with induction of RAD51 nuclear foci as assessed by immunohistochemistry and clinical activity of rucaparib with a greater circulating tumour DNA suppression after 15 days of treatment (n = 15; p = 0.027). A phase II trial is currently evaluating olaparib associated with trastuzumab in HER2+ mBC that present either a *gBRCA1/2* mutation or an HRDetect + tumour (NCT039311551). In the RUBY study, 2 patients with high genomic LOH were predicted to have *BRCA1/2* deficiency with HRDetect (>0.7), including 1 patient, that presented a complete response and was still under treatment with a duration of response >28.5 months and 1 patient with a partial response. The patient with an important partial liver metastasis response presented a higher HRDetect score than most non-responders (0.46). Only one patient with high genomic LOH and prolonged stable disease (>12 months) presented a low HRDetect score (0.02). Recently, a new genome-wide mutational scar-based test, CHORD (Classifier of Homologous Recombination Deficiency) has also been developed to predict HRD without data published regarding its predictive value of response to DNA crosslinking agents of PARP inhibitors [50]. However, WGS-based algorithms require the acquisition and analysis of large and complex sequence data. Analysing WGS is a highly time and resources consuming process, which requires important bioinformatics expertise in addition to the adapted infrastructure for long term storage. Altogether, analytical steps to obtain an

HRDetect score represent a limitation to daily clinical practice at the present time.

Conclusion

Overall, this study shows that a small subset of patients with HRD but no *gBRCA1/2* mutation could benefit from PARP inhibition. Nevertheless, currently available testing is probably too broad, and there is a need to develop additional biomarkers to identify selectively potential responders. HRDetect and specific mutations on HR pathways (*gPALB2*, *sBRCA1/2* mutations) are candidate biomarkers. A further meta-analysis of current phase II trials will help better define which biomarker combination may accurately predict response to PARP inhibitors in patients without *gBRCA1/2* mutations.

Author contributions

A.P and F.A. designed the study, contributed to the writing of the paper and were involved in recruitment, clinical care and data collection.

O.T., M.A., M.P.S., A.G., C.V. were involved in recruitment, clinical care and data collection.

P.J. contributed to the microarray data formatting.

A.L. calculated the LOH score for all data sets and determined the LOH cutoff score.

T.F. supervised biostatistical analysis regarding prognostic value of LOH score.

V.S. supervised all statistical analyses related to outcomes.

D.N. performed the central review of all study CT-scans.

K.D. was in charge of the whole genome bioinformatics analyses.

I.B. and C.C. revised the value of the BRCA mutations for patient's inclusions.

N.D. processed samples and generated the whole genome sequences.

M.J. is the project manager of the RUBY and SAFIR02-breast/SAFIR-TOR trials and centralised biological samples and data.

All authors approved the final manuscript and contributed to critical revisions of its intellectual content.

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Conflict of interest statement

Fabrice André received research fundings and served as speaker/advisor (compensated to the hospital) for Roche, AstraZeneca, Daiichi Sankyo, Pfizer, Novartis, Lilly.

Anne PATSOURIS received consulting fees (e.g. advisory boards) and served as a speaker (both compensated to the hospital) for Pfizer, Lilly, received travel fees from Roche, Esai, Amgen, Pfizer.

Olivier TREDAN received research grant from Roche, MSD-Merck, BMS, personal fees from Roche, MSD-Merck, Pfizer, Lilly, Astrazeneca, Sandoz, Novartis-Sandoz, Daiichi Sankyo and travel fees from Eli-Lilly, Pfizer, Novartis, Astra Zeneca.

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Anthony GONCALVES received research fundings and served as speaker/advisor, compensated to the hospital for Astra Zeneca, Pfizer, Novartis, Roche, MSD, Lilly.

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The personal link of interests of each author not related to the RUBY study.

Andrea LOEHR is affiliated to Clovis and a no competing interest to disclose otherwise.

Marie-Paule SABLIN received personal fees from Servier.

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Appendix C. Supplementary data

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References

- [1] Grinda T, Antoine A, Jacot W, Blaye C, Cottu P-H, Diéras, et al. Evolution of overall survival and receipt of new therapies by subtype among 20446 metastatic breast cancer patients in the 2008–2017ESME cohort. *ESMO Open* 2021;6(3):100114. <https://doi.org/10.1016/j.esmoop.2021.100114>.
- [2] Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature* 2012;490(7418):61–70. <https://doi.org/10.1038/nature11412>.
- [3] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144(5):646–74. <https://doi.org/10.1016/j.cell.2011.02.013>.
- [4] Farmer Hannah, McCabe Nuela, Lord Christopher J, Tutt Andrew NJ, Johnson Damian A, Richardson Manuela, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 2005;434(7035):917–21. <https://doi.org/10.1038/nature03445>.
- [5] Lord CJ, Ashworth A. PARP inhibitors: synthetic lethality in the clinic. *Science* 2017;355(6330):1152–8. <https://doi.org/10.1126/science.aam7344>.
- [6] Research C for DE and. FDA approves olaparib for germline BRCA-mutated metastatic breast cancer. FDA; 2019. Published online April 16, <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-olaparib-germline-brca-mutated-metastatic-breast-cancer>. [Accessed 19 April 2020].
- [7] Research C for DE and. FDA approves talazoparib for gBRCAm HER2-negative locally advanced or metastatic breast cancer. FDA; 2019. Published online December 20, <https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-talazoparib-gbrcam-her2-negative-locally-advanced-or-metastatic-breast-cancer>. [Accessed 19 April 2020].
- [8] Robson M, Im S-A, Senkus E, Xu B, Domchek S-M, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *N Engl J Med* 2017;377(6):523–33. <https://doi.org/10.1056/NEJMoa1706450>.
- [9] Litton JK, Rugo HS, Ettl J, Hurvitz S, Gonçalves A, Lee K-H, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. *N Engl J Med* 2018;379(8):753–63. <https://doi.org/10.1056/NEJMoa1802905>.
- [10] Gelmon KA, Fasching PA, Couch FJ, Balmaña J, Delalogue S, Labidi-Galy I, et al. Clinical effectiveness of olaparib monotherapy in germline BRCA-mutated, HER2-negative metastatic breast cancer in a real-world setting: phase IIIb LUCY interim analysis. *Eur J Cancer Oxf Engl* 1990;152:68–77. <https://doi.org/10.1016/j.ejca.2021.03.029>. 2021.
- [11] Diéras V, Han HS, Kaufman B, Wildiers H, Friedlander M, Ayoub E-H, et al. Veliparib with carboplatin and paclitaxel in BRCA-mutated advanced breast cancer (BROCADE3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2020;21(10):1269–82. [https://doi.org/10.1016/S1470-2045\(20\)30447-2](https://doi.org/10.1016/S1470-2045(20)30447-2).
- [12] Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, et al. Adjuvant olaparib for patients with BRCA1- or BRCA2-mutated breast cancer. *N Engl J Med* 2021;384(25):2394–405. <https://doi.org/10.1056/NEJMoa2105215>.
- [13] Malone KE, Daling JR, Doody DR, Hsu L, Bernstein L, Coates R-J, et al. Prevalence and predictors of BRCA1 and BRCA2 mutations in a population-based study of breast cancer in white and black American women ages 35 to 64 years. *Cancer Res* 2006;66(16):8297–308. <https://doi.org/10.1158/0008-5472.CAN-06-0503>.
- [14] Bertucci F, Ng CKY, Patsouris A, Droin N, Piscuoglio S, Carbuca N, et al. Genomic characterization of metastatic breast cancers. *Nature* 2019;569(7757):560–4. <https://doi.org/10.1038/s41586-019-1056-z>.
- [15] Turner N, Tutt A, Ashworth A. Hallmarks of “BRCAness” in sporadic cancers. *Nat Rev Cancer* 2004;4(10):814–9. <https://doi.org/10.1038/nrc1457>.
- [16] Birkbak NJ, Wang ZC, Kim J-Y, Eklund A-C, Li Q, Tian R, et al. Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. *Cancer Discov* 2012;2(4):366–75. <https://doi.org/10.1158/2159-8290.CD-11-0206>.
- [17] Abkevich V, Timms KM, Hennessy BT, Potter J, Carey M-S, Myer L-A, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br J Cancer* 2012;107(10):1776–82. <https://doi.org/10.1038/bjc.2012.451>.
- [18] Popova T, Manié E, Rieunier G, Caux-Moncoutier V, Tirapo C, Dubois T, et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. *Cancer Res* 2012;72(21):5454–62. <https://doi.org/10.1158/0008-5472.CAN-12-1470>.
- [19] Alexandrov LB, Nik-Zainal S, Wedge DC, Campbell PJ, Stratton MR. Deciphering signatures of mutational processes operative in human cancer. *Cell Rep* 2013;3(1):246–59. <https://doi.org/10.1016/j.celrep.2012.12.008>.
- [20] Davies H, Glodzik D, Morganella S, Yates L-R, Staaf J, Zou X, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat Med* 2017;23(4):517–25. <https://doi.org/10.1038/nm.4292>.
- [21] Gruber JJ, Afghahi A, Hatton A, Scott D, McMillan A, Ford J-M, et al. Talazoparib beyond BRCA: a phase II trial of talazoparib monotherapy in BRCA1 and BRCA2 wild-type patients with advanced HER2-negative breast cancer or other solid tumours with a mutation in homologous recombination (HR) pathway genes. *J Clin Oncol* 2019;37(15_suppl):3006. https://doi.org/10.1200/JCO.2019.37.15_suppl.3006. 3006.
- [22] Tung NM, Robson ME, Venz S, Santa-Maria C-A, Nanda R, Marcom P-K, et al. TBCRC 048: phase II study of olaparib for metastatic breast cancer and mutations in homologous recombination-related genes. *J Clin Oncol Off J Am Soc Clin Oncol* 2020;38(36):4274–82. <https://doi.org/10.1200/JCO.20.02151>.
- [23] Kristeleit R, Shapiro GI, Burris HA, Oza A-M, LoRusso P, Patel M-R, et al. A phase I-II study of the oral poly(ADP-ribose) polymerase inhibitor rucaparib in patients with germline BRCA1/2-mutated ovarian carcinoma or other solid tumours. *Clin Cancer Res Off J Am Assoc Cancer Res* 2017. <https://doi.org/10.1158/1078-0432.CCR-16-2796>. Published online March 6.
- [24] Abida W, Campbell D, Patnaik A, Shapiro J-D, Sautois B, Vogelzang N, et al. Non-BRCA DNA damage repair gene alterations and response to the PARP inhibitor rucaparib in metastatic castration-resistant prostate cancer: analysis from the phase II TRITON2 study. *Clin Cancer Res Off J Am Assoc Cancer Res* 2020. <https://doi.org/10.1158/1078-0432.CCR-20-0394>. Published online February 21.
- [25] Balasubramaniam S, Beaver JA, Horton S, Fernandes L-L, Tang S, Horne H-N, et al. FDA approval summary: rucaparib for the treatment of patients with deleterious BRCA mutation-associated advanced ovarian cancer. *Clin Cancer Res Off J Am Assoc Cancer Res* 2017;23(23):7165–70. <https://doi.org/10.1158/1078-0432.CCR-17-1337>.
- [26] Abida W, Campbell D, Patnaik A, Shapiro J, Vogelzang N-J, Bryce A-H, et al. 846PD - preliminary results from the TRITON2 study of rucaparib in patients (pts) with DNA damage repair (DDR)-deficient metastatic castration-resistant prostate cancer (mCRPC): updated analyses. *Ann Oncol* 2019;30:v327–8. <https://doi.org/10.1093/annonc/mdz248.003>.

- [27] Oza AM, Tinker AV, Oaknin A, Shapira-Frommer R, McNeish I-A, Swisher E-M, et al. Antitumour activity and safety of the PARP inhibitor rucaparib in patients with high-grade ovarian carcinoma and a germline or somatic BRCA1 or BRCA2 mutation: integrated analysis of data from Study 10 and ARIEL2. *Gynecol Oncol* 2017;147(2):267–75. <https://doi.org/10.1016/j.ygyno.2017.08.022>.
- [28] Swisher EM, Lin KK, Oza AM, Scott C-L, Giordano H, Sun J, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2017;18(1):75–87. [https://doi.org/10.1016/S1470-2045\(16\)30559-9](https://doi.org/10.1016/S1470-2045(16)30559-9).
- [29] Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing S-R, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol* 2013;31(11):1023–31. <https://doi.org/10.1038/nbt.2696>.
- [30] Massard C, Michiels S, Féré C, Le Deley M-C, Lacroix L, Hollebecque A, et al. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 trial. *Cancer Discov* 2017;7(6):586–95. <https://doi.org/10.1158/2159-8290.CD-16-1396>.
- [31] Bachelot T, Filleron T, Bieche I, Arnedos M, Campone M, Dalenc F, et al. Durvalumab compared to maintenance chemotherapy in metastatic breast cancer: the randomized phase II SAFIR02-BREAST IMMUNO trial. *Nat Med* 2021;27(2):250–5. <https://doi.org/10.1038/s41591-020-01189-2>.
- [32] Van Loo P, Nordgard SH, Lingjærde OC, Russnes H-G, Rye I-H, Sun W, et al. Allele-specific copy number analysis of tumours. *Proc Natl Acad Sci U S A* 2010;107(39):16910–5. <https://doi.org/10.1073/pnas.1009843107>.
- [33] Addressing Aneuploidy and Nonaberrant Cell Admixture in Tumour Samples for Copy Number Variation (CNV) and Loss of Heterozygosity (LOH) Analysis. Accessed August 25, 2020. <http://resources.biodiscovery.com/white-papers/aneuploidy-and-nonaberrant-cell-admixture-in-tumour-samples>.
- [34] Thiagalingam S, Laken S, Willson JK, Markowitz S-D, Kinzler K-W, Vogelstein B, et al. Mechanisms underlying losses of heterozygosity in human colorectal cancers. *Proc Natl Acad Sci U S A* 2001;98(5):2698–702. <https://doi.org/10.1073/pnas.051625398>.
- [35] André F, Bachelot T, Commo F, Campone M, Arnedos M, Diéras V, et al. Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER). *Lancet Oncol* 2014;15(3):267–74. [https://doi.org/10.1016/S1470-2045\(13\)70611-9](https://doi.org/10.1016/S1470-2045(13)70611-9).
- [36] Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski E-M, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res* 2001;29(1):308–11. <https://doi.org/10.1093/nar/29.1.308>.
- [37] Patsouris A, Filleron T, Jacquet A, Gonçalves A, Bonnefoi H, Letourneau C, et al. Abstract PD8-12: mutational processes, genome evolution and outcome in metastatic breast cancers. *Cancer Res* 2018;78(4 Supplement):PD8–12. <https://doi.org/10.1158/1538-7445.SABCS17-PD8-12>.
- [38] Razavi P, Chang MT, Xu G, Bandlamudi C, Ross D-S, Vasan N, et al. The genomic landscape of endocrine-resistant advanced breast cancers. *Cancer Cell* 2018;34(3):427–38. <https://doi.org/10.1016/j.ccell.2018.08.008>. e6.
- [39] Gelmon KA, Tischkowitz M, Mackay H, Swenerton K, Robidoux A, Tonkin K, et al. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. *Lancet Oncol* 2011;12(9):852–61. [https://doi.org/10.1016/S1470-2045\(11\)70214-5](https://doi.org/10.1016/S1470-2045(11)70214-5).
- [40] Riaz N, Blecua P, Lim RS, Shen R, Higginson D-S, Weinhold N, et al. Pan-cancer analysis of bi-allelic alterations in homologous recombination DNA repair genes. *Nat Commun* 2017;8(1):857. <https://doi.org/10.1038/s41467-017-00921-w>.
- [41] Meeting Library | TBCRC 048: A phase II study of olaparib monotherapy in metastatic breast cancer patients with germline or somatic mutations in DNA damage response (DDR) pathway genes (Olaparib Expanded). Accessed May 27, 2020. <https://meetinglibrary.asco.org/record/185171/abstract>.
- [42] Eikesdal HP, Yndestad S, Elzawahry A, Llop-Guevara A, Gilje B, Blix E-S, et al. Olaparib monotherapy as primary treatment in unselected triple negative breast cancer. *Ann Oncol Off J Eur Soc Med Oncol* 2021;32(2):240–9. <https://doi.org/10.1016/j.annonc.2020.11.009>.
- [43] Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Lond Engl* 2017;390(10106):1949–61. [https://doi.org/10.1016/S0140-6736\(17\)32440-6](https://doi.org/10.1016/S0140-6736(17)32440-6).
- [44] Polak P, Kim J, Braunstein LZ, Karlic R, Haradhvala N-J, Tia G, et al. A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. *Nat Genet* 2017;49(10):1476–86. <https://doi.org/10.1038/ng.3934>.
- [45] Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman C-D, Raine K, et al. Mutational processes molding the genomes of 21 breast cancers. *Cell* 2012;149(5):979–93. <https://doi.org/10.1016/j.cell.2012.04.024>.
- [46] Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature* 2016;534(7605):47–54. <https://doi.org/10.1038/nature17676>.
- [47] Morganello S, Alexandrov LB, Glodzik D, Zou X, Davies H, Staaf J, et al. The topography of mutational processes in breast cancer genomes. *Nat Commun* 2016;7:11383. <https://doi.org/10.1038/ncomms11383>.
- [48] Staaf J, Glodzik D, Bosch A, Vallon-Christersson J, Reuterswärd C, Häkkinen J, et al. Whole-genome-sequencing of triple negative breast cancers in a population-based clinical study. *Nat Med* 2019;25(10):1526–33. <https://doi.org/10.1038/s41591-019-0582-4>.
- [49] Chopra N, Tovey H, Pearson A, Cutts R, Toms C, Proszek P, et al. Homologous recombination DNA repair deficiency and PARP inhibition activity in primary triple negative breast cancer. *Nat Commun* 2020;11(1):2662. <https://doi.org/10.1038/s41467-020-16142-7>.
- [50] Nguyen L, Martens J WM, Van Hoeck A, Cuppen E. Pan-cancer landscape of homologous recombination deficiency. *Nat Commun* 2020;11(1):5584. <https://doi.org/10.1038/s41467-020-19406-4>.