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Emergence of BRCA Reversion Mutations in Patients with Metastatic Castration-resistant Prostate Cancer After Treatment with Rucaparib

Andrea Loehr[a], *Arif Hussain*[b], *Akash Patnaik*[c], *Alan H. Bryce*[d], *Daniel Castellano*[e], *Albert Font*[f], *Jeremy Shapiro*[g], *Jingsong Zhang*[h], *Brieuc Sautois*[i], *Nicholas J. Vogelzang*[j], *Gurkamal Chatta*[k], *Kevin Courtney*[l], *Andrea Harzstark*[m], *Francesco Ricci*[n], *Darrin Despain*[o], *Simon Watkins*[p], *Charmin King*[q], *Minh Nguyen*[a], *Andrew D. Simmons*[a], *Simon Chowdhury*[r,s], *Wassim Abida*[t,*]

[a] *Translational Medicine, Clovis Oncology, Inc, Boulder, CO, USA;* [b] *Greenebaum Comprehensive Cancer Center, University of Maryland, Baltimore, MD, USA;* [c] *Section of Hematology/Oncology, Department of Medicine, University of Chicago, Chicago, IL, USA;* [d] *Hematology/Oncology, Mayo Clinic, Phoenix, AZ, USA;* [e] *Medical Oncology Department, Hospital Universitario 12 de Octubre, Madrid, Spain;* [f] *Medical Oncology, Hospital Universitari Germans Trias i Pujol, Barcelona, Spain;* [g] *Medical Oncology, Cabrini Hospital, Malvern, VIC, Australia;* [h] *Genitourinary Oncology, H. Lee Moffitt Cancer Center, Tampa, FL, USA;* [i] *Medical Oncology, CHU Sart Tilman, University of Liège, Liège, Belgium;* [j] *Medical Oncology, Comprehensive Cancer Centers of Nevada, Las Vegas, NV, USA;* [k] *Medical Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA;* [l] *Hematology/Oncology, UT Southwestern Medical Center, Dallas, TX, USA;* [m] *Hematology/Oncology, Kaiser Permanente Oncology Clinical Trials Program, San Francisco, CA, USA;* [n] *Department of Drug Development and Innovation (D3i), Institut Curie, Paris, France;* [o] *Biostatistics, Clovis Oncology, Inc., Boulder, CO, USA;* [p] *Clinical Development, Clovis Oncology UK Ltd, Cambridge, UK;* [q] *Clinical Operations, Clovis Oncology, Inc, Boulder, CO, USA;* [r] *Medical Oncology, Guy's Hospital, London, UK;* [s] *Sarah Cannon Research Institute, London, UK;* [t] *Genitourinary Oncology Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

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Abstract

Background: Poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors are approved in the USA for the treatment of patients with *BRCA1* or *BRCA2* (BRCA) mutated (BRCA+) metastatic castration-resistant prostate cancer (mCRPC). BRCA reversion mutations are a known mechanism of acquired resistance to PARP inhibitors in multiple cancer types, although their impact and prevalence in mCRPC remain unknown.
Objective: To examine the prevalence of BRCA reversion mutations in the plasma of patients with BRCA+ mCRPC after progression on rucaparib.
Design, setting, and participants: Men with BRCA+ mCRPC enrolled in Trial of Rucaparib in Prostate Indications 2 (TRITON2) were treated with rucaparib after progressing on one to two lines of androgen receptor–directed and one taxane-based therapy. Cell-free DNA from the plasma of 100 patients, collected at the end of treatment after confirmed progression before May 5, 2020, was queried for BRCA reversion mutations using next-generation sequencing (NGS).
Outcome measurements and statistical analysis: The association of clinical efficacy and postprogression genomics was measured in 100 patients with BRCA+ mCRPC treated with rucaparib.
Results and limitations: No baseline BRCA reversion mutations were observed in 100 BRCA+ patients. NGS identified somatic BRCA reversion mutations in 39% (39/100) of patients after progression. Reversion rates were similar for *BRCA2* and *BRCA1*,

* Corresponding author. Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. Tel. +1 646-422-4633; Fax: +1 646-227-2417.
E-mail address: abidam@mskcc.org (W. Abida).

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irrespective of germline or somatic status, but higher in samples with a high tumor DNA fraction. Most patients with reversions (74%, 29/39) had two or more reversion mutations occurring subclonally at lower allele frequencies than the original BRCA mutations. The incidence of BRCA reversion mutations increased with the duration of rucaparib treatment. The frequency of reversion mutations was higher in patients with an objective (58%) or a prostate-specific antigen (69%) response compared with those without either (39% and 29%, respectively).

Conclusions: These findings suggest that BRCA reversion mutations are a significant mechanism of acquired resistance to rucaparib in patients with BRCA+ mCRPC, with evidence of subclonal convergence promoting systemic resistance.

Patient summary: Men with BRCA mutated metastatic castration-resistant prostate cancer enrolled in TRITON2 were treated with rucaparib after progressing on one to two lines of androgen receptor–directed and one taxane-based therapy. Cell-free DNA from the plasma of 100 patients, collected after radiographic or prostate-specific antigen progression before May 5, 2020, was analyzed by next-generation sequencing and queried for BRCA reversion mutations.

1. Introduction

The poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitor (PARPi) rucaparib is approved in the USA for the treatment of patients with BRCA-mutated metastatic castration-resistant prostate cancer (mCRPC), based on the results from the Trial of Rucaparib in Prostate Indications 2 (TRITON2) study [1].

Protein-inactivating germline or somatic BRCA alterations are observed in approximately 7.3–12% of men with advanced prostate cancer [2–5] and have been identified as drivers of the disease [6], making them ideal candidates for PARPi treatment. Secondary BRCA mutations within the mutated BRCA allele, restoring the open reading frame (ORF) and re-establishing protein function, have been described in patients with mCRPC [7–10], and breast or ovarian cancer [11–13] after PARPi or platinum-based chemotherapy. Reversion mutations of both germline and somatic BRCA alterations have been observed [7] in the form of small deletions, single-nucleotide variants (SNVs) [14], large rearrangements and deletions bypassing the original alteration [15], or splice site mutations causing exon skipping [16]. By these mechanisms, they reinstate homologous repair capability and promote drug resistance. Other proposed mechanisms of resistance to PARPi include loss of PARP1 function, epigenetic changes, or restoration of ADP-ribosylation [17–21].

While BRCA reversion mutations have been studied in sizeable cohorts of ovarian cancer [11], reports in patients with prostate cancer occur primarily in the form of case studies [5,8–10,22–25], and the primary mechanism of resistance to PARPi in *BRCA*-mutated prostate cancer remains unclear. To date, there is no reported study in a large cohort of patients with mCRPC evaluating the prevalence of BRCA reversion mutations and their relationship with patient demographics and clinical efficacy. Here, we present analyses of postprogression cell-free DNA (cfDNA) samples from a cohort of 100 patients with BRCA-mutated mCRPC treated with rucaparib to evaluate the prevalence of BRCA reversion mutations as a mechanism of acquired resistance to PARPi in mCRPC.

2. Patients and methods

2.1. Study description

TRITON2 was an international, open-label, phase 2 study evaluating rucaparib in patients with mCRPC associated with DNA damage repair (DDR) deficiency. Men aged ≥18 yr with histologically or cytologically confirmed mCRPC, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate organ function were enrolled. Eligible patients had a known deleterious germline or somatic alteration in *BRCA1*, *BRCA2*, or one of 13 other DDR genes identified by various assays [1] and disease progression following one to two lines of next-generation androgen receptor–directed therapy for prostate cancer and one prior taxane-based chemotherapy for castration-resistant disease. Patients treated with a PARPi, mitoxantrone, cyclophosphamide, or platinum-based chemotherapy, or with an active secondary malignancy were excluded. Patients were enrolled irrespective of measurable disease status.

The primary endpoint was the confirmed objective response rate (ORR) by a blinded independent radiology review as per the modified RECIST 1.1 [26] and PCWG 3 [27] criteria in patients with measurable disease. A confirmed prostate-specific antigen (PSA) response (≥50% decrease from baseline confirmed by a second measurement ≥3 wk later) in all patients was a secondary endpoint. Duration of response was defined as the time from the date of the first confirmed response to the date progression was first documented plus 1 d, and summarized using the Kaplan-Meier methodology.

The study was approved by national or local institutional review boards, and performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Patients provided written informed consent before participation.

Plasma samples were collected prior to rucaparib treatment on day 1 of cycle 1 and during every 28-d cycle thereafter until the end of trial participation, including at the end of treatment (EOT), which was typically at radiographic progression or clinical progression at investigator discretion.

2.2. Genomic next-generation sequencing assays

The Guardant360 assay [28,29] was used for next-generation sequencing (NGS) of cfDNA extracted from plasma (Supplementary material). A BRCA reversion was defined as a base substitution changing a nonsense mutation to a nondeleterious missense mutation, an insertion or dele-

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tion (indel) restoring the ORF disrupted by the deleterious mutation, or a small or large intragenic deletion removing a deleterious SNV/small indel entirely. For an indel to be classified as a reversion, the combined effect of the primary and secondary mutations was required to result in a nucleotide change that restored the ORF.

The Color Hereditary Cancer Test [30] was used to determine the germline status of baseline BRCA alterations.

3. Results

3.1. Patients

By May 5, 2020, 114 men with BRCA+ mCRPC enrolled in TRITON2 had either a confirmed radiographic (80%, 91/114) or a clinical (20%, 23/114) progression leading to the discontinuation of rucaparib treatment. EOT plasma samples were collected after progression and within 44 d of EOT for all patients. A total of 23 of 114 (20%) patients had BRCA alteration types (homozygous deletions and rearrangements) for which no known mechanism of reversion has been reported; an EOT sample was sequenced for all 23 patients. In addition, 91 (80%, 91/114) patients had short protein truncation indels, deleterious SNVs, and splice site alterations. EOT plasma samples were available for 93% (85/91) of these patients of whom one failed NGS. The baseline BRCA mutation was not detected in the EOT samples of six patients, mainly due to a low (<1%) allele frequency (AF) of the original alteration detected in plasma or low circulating tumor DNA (ctDNA) content in the EOT sample. These samples and one without detectable tumor DNA were excluded from analysis. In the final cohort of 100 patients with BRCA+ mCRPC and postprogression NGS data, 77 had alterations in which mutations could potentially restore BRCA function, and 23 had alteration types with no known or reported mechanism of restoring BRCA function (Fig. 1).

3.2. Baseline patient demographics and genomics

Patient demographics were similar in patients who developed and those who did not develop reversion mutations, including prognostic factors such as age, time since diagnosis, ECOG status, PSA at baseline, and measurable disease status (Table 1).

The majority of BRCA mutations were somatic (54%, 54/100) versus germline (46%, 46/100), with 14% (14/100) occurring in *BRCA1* and 86% in *BRCA2* (86/100). The distribution of alteration types was as follows: frameshift (53%, 53/100), nonsense (16%, 16/100), missense (6%, 6/100), splicing (2%, 2/100) variants, homozygous loss (14%, 14/100), and rearrangements (9%, 9/100). All patients

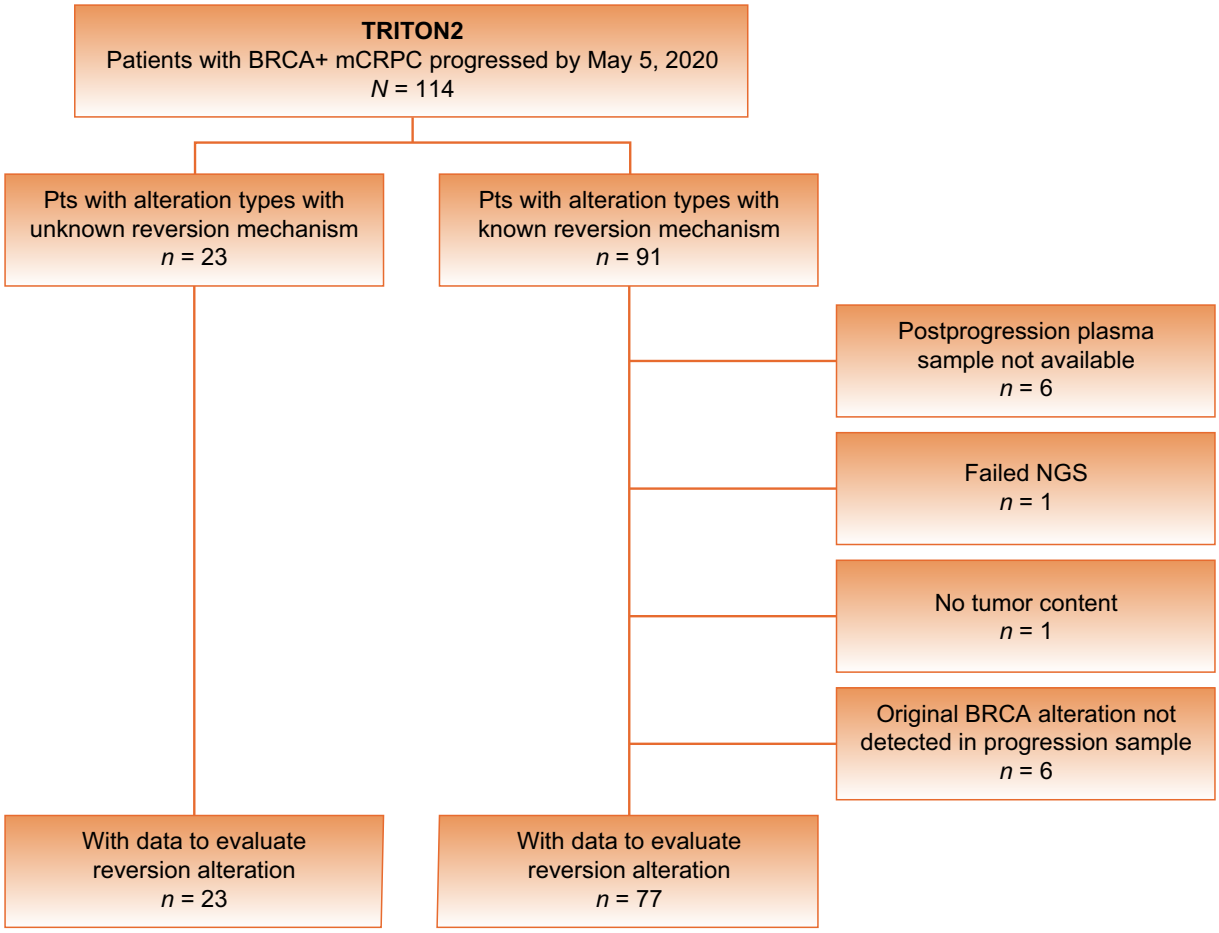

Fig. 1 – Summary of patients included in the analysis of reversion mutations in postprogression plasma samples. Alterations with unknown mechanisms of reversion were homozygous deletions and rearrangements. BRCA+ = *BRCA1* or *BRCA2* mutated; mCRPC = metastatic castration-resistant prostate cancer; NGS = next-generation sequencing; Pts = patients; TRITON2 = Trial of Rucaparib in Prostate Indications 2.

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Table 1 – Patient baseline characteristics prior to treatment with rucaparib

Baseline characteristics	All patients (*N* = 100)	Patients with nonreversible BRCA mutations at baseline (*n* = 23)	Patients with potentially reversible BRCA mutations at baseline (*n* = 77)	Patients with BRCA reversion mutations after PARPi treatment (*n* = 39)	Patients without BRCA reversion mutations after PARPi treatment (*n* = 38)
Age (yr), median (IQR)	72 (65–76)	75 (69–79)	70 (65–75)	70 (65–74)	71 (62–76)
Time since cancer diagnosis (yr), median (IQR)	4.2 (2.4–9.1)	3.9 (2.7–10.0)	4.5 (2.2–9.0)	4.2 (2.1–9.0)	5.1 (2.4–9.3)
ECOG PS, no. (%)					
0	31 (31)	4 (17)	27 (35)	14 (36)	13 (34)
1	67 (67)	18 (78)	49 (64)	24 (62)	25 (66)
≥2	2 (2)	1 (4)	1 (1)	1 (3)	0 (0)
Baseline PSA (ng/ml), median (IQR)	87.1 (29.8–311.2)	162 (59.8–415.0)	65.3 (28.9–211.2)	92.1 (43.6–321.1)	44.3 (22.1–135.0)
ISUP grade group ≥4, no. (%)	71 (71)	16 (70)	55 (71)	30 (77)	25 (66)
Measurable disease, no. (%)	52 (52)	12 (52)	40 (52)	18 (46)	22 (58)
Visceral and nodal disease	20 (20)	7 (30)	13 (17)	5 (13)	8 (21)
Only nodal disease	32 (32)	5 (22)	27 (35)	13 (33)	14 (37)
Nonmeasurable disease, no. (%)	48 (48)	11 (48)	37 (48)	21 (54)	16 (42)
Bone-only disease	31 (31)	8 (35)	23 (30)	14 (36)	9 (24)
Other	17 (17)	3 (13)	14 (18)	7 (18)	7 (18)

ECOG PS = Eastern Cooperative Oncology Group performance status; IQR = interquartile range; PARPi = poly(adenosine diphosphate-ribose) polymerase inhibitor; PSA = prostate-specific antigen.

had somatic testing before treatment, and no baseline BRCA reversion mutations were observed.

3.3. *Postprogression patient genomics*

After confirmed progression on rucaparib, EOT plasma was collected and interrogated for secondary BRCA mutations. In contrast to the pretreatment genomics, plasma samples taken at EOT identified BRCA reversion mutations in 39% (39/100) of all patients with BRCA+ mCRPC (Supplementary material).

No secondary BRCA mutations were observed in 23 samples from patients with homozygous deletions or rearrangements.

All BRCA reversion mutations were detected in the cohort of 77 patients with reversible BRCA alterations at baseline. A total of 189 reversion mutations were observed across 39 patients. Most patients (74%, 29/39) with a BRCA reversion developed two or more secondary alterations, with the observed maximum being 12 (Fig. 2A). Most reversion mutations (87%, 165/189) were short indels with point mutations causing the restoration of the ORF in 13% (24/189) of cases.

The median ctDNA fraction in the plasma samples as estimated by the maximum somatic AF was 14.5% (interquartile range [IQR] 3.2–27.8%). Reversion mutations were detected in 66% (25/38) of samples with higher than median ctDNA fractions compared with 36% (14/39) among samples with lower than median ctDNA fractions.

Patients developed reversion mutations at similar frequencies: (1) across both BRCA genes, (2) whether the original BRCA mutations were germline or somatic in origin, and (3) regardless of the original alteration types (Table 2), although some subgroups were small. Reversion of splice site mutations (*n* = 2) was not observed.

The original deleterious *BRCA1* and *BRCA2* alterations were distributed across the genes, and their reversion did not depend on gene location: there was no preferred location of reverted mutations along the genes (Supplementary Fig. 1).

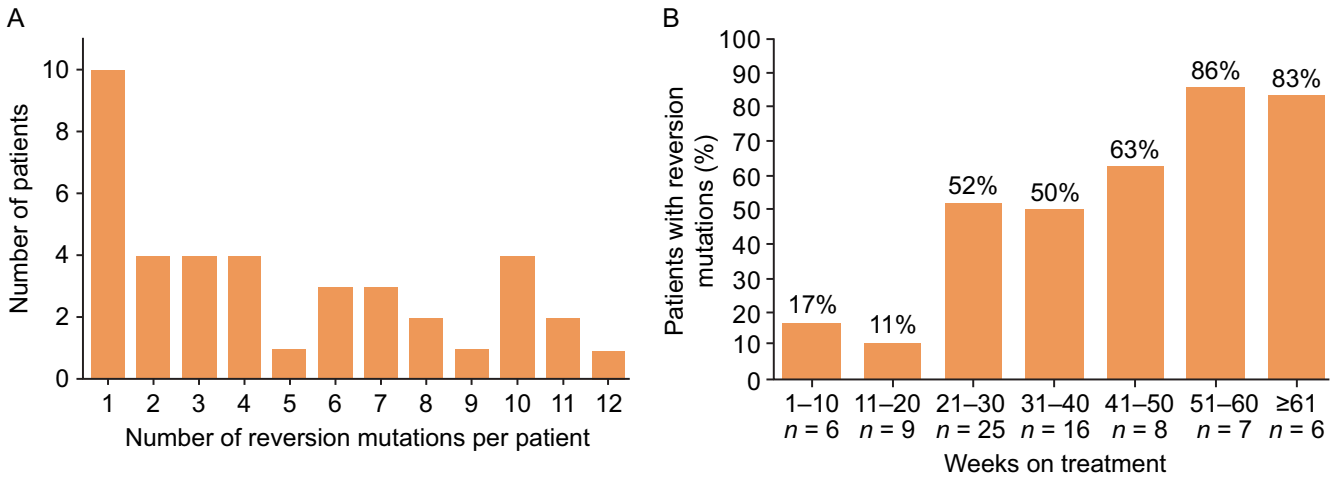

Fig. 2 – Summary of BRCA reversion mutation incidence. (A) Number of reversion mutations detected per patient. (B) Frequency of patients who developed BRCA reversion mutations within intervals of increasing treatment duration. BRCA indicates *BRCA1* or *BRCA2*.

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Table 2 – Patients with reversion mutations across the *BRCA* genes, germline status, and alteration type

	Patients with BRCA+ mCRPC with a reversion alteration, *n*/*N* (%)
All patients	39/100 (39)
Alteration type	
Frameshift mutation	30/53 (57)
Nonsense mutation	6/16 (38)
Missense mutation	2/6 (33)
Splice site mutation	0/2 (0)
Homozygous deletion	0/14 (0)
Rearrangement	0/9 (0)
Patients with reversible alteration types (excl. homozygous deletions, rearrangements)	
Gene	
BRCA1	3/7 (43)
BRCA2	36/70 (51)
Germline status	
Germline	26/46 (57)
Somatic	13/31 (42)

mCRPC = metastatic castration-resistant prostate cancer.

The median AF of the detected reversion mutations was 0.6% (IQR 0.2–1.9%), and reversion mutation AFs were lower than the AFs of the original deleterious mutations in all samples (Supplementary Fig. 2). In samples with multiple reversion mutations, individual dominant subclones with significantly higher AFs than any other reversion mutations were not observed regularly.

Notably, two patients each had two different deleterious *BRCA2* alterations at baseline. After progression, each deleterious baseline *BRCA2* alteration was reverted by two sets of one or more separate reversion clones (Supplementary Table 1).

3.4. Demographics and clinical outcome in patients with or without detectable BRCA reversion mutations

No differences in baseline demographic characteristics were observed between patients with and without reversion mutations (Table 1).

The incidence rate of reversion mutations increased with time on treatment (Fig. 2B). Logistic regression showed that the odds of developing a reversion mutation increased by approximately 31% for each additional month on study drug (odds ratio = 1.31, 95% confidence interval [CI] 1.11, 1.55, $p = 0.001$). The number of reversion mutations developed per patient did not change with time on treatment.

The median (IQR) time on treatment for patients with detected reversion mutations was 8.2 (5.9, 11.7) mo compared with 5.3 (3.7, 8.1) mo in patients without such mutations (hazard ratio [HR] = 0.43, 95% CI 0.27–0.60, $p < 0.001$; Fig. 3A). A total of 40 patients within the analysis group had measurable disease at baseline and were ORR evaluable. A total of 12 patients with measurable disease had a radiographic response, and a higher percentage of patients who responded had developed detectable reversion mutations (58%, 95% CI 25.1–80.8, 7/12) than patients who did not have a radiographic response (39%, 11/28, $p = 0.4$).

Among all 77 patients, 42 had a confirmed PSA response, and a greater proportion of patients in the group with a PSA response (69%, 29/42) developed detectable reversion mutations than in the group of 35 patients without a PSA response (29%, 10/35, $p = 0.066$). Overall, the median (IQR) duration of PSA response was 3.7 (2.8, 5.5) mo and was only slightly different between patients with (3.7 [2.9, 4.7]) and without (3.8 [2.3, 6.4]) detected reversion mutations (HR = 0.93, 95% CI 0.46–1.87, $p = 0.8$; Fig. 3B). All but one patient with a reversion mutation had reductions of >30% in PSA level from baseline, and the median best change in PSA level from baseline was larger in patients with (−76%; IQR −83% to −56%) than in patients without (−40%; IQR −68% to 12%) reversion mutations ($p < 0.001$; Supplementary Fig. 3).

3.5. Longitudinal case study of patients with BRCA+ mCRPC and emergent reversion mutations

A 62-yr-old mCRPC patient with the *BRCA2* founder mutation 6174delT (S1982fs) was treated with rucaparib 7 yr after his initial diagnosis of metastatic prostate cancer. At the initiation of treatment, the patient had metastatic disease to the liver and lymph nodes as well as extensive bone disease. The patient received rucaparib for 71 wk from September 2017 until February 2019.

A radiographic partial response was recorded after 8 wk on rucaparib treatment and was maintained for 52 wk. Over the course of treatment, longitudinal plasma samples were sequenced, and the emergence of 11 different subclonal reversion mutations was observed (Fig. 4A). The first subclonal *BRCA2* reversion mutation was detected at an AF of 0.2% at 33 wk on treatment, prior to the patient's radiographic complete response (CR) at 61 wk on treatment. At the time of the CR, the patient's plasma showed six different reversion clones, with the most prevalent one at an AF of 0.71%. The patient continued on treatment for another 4 mo despite rising PSA, due to clinical benefit. At the EOT (due to radiographic liver progression), the cfDNA sample displayed six reversion mutation clones, with the most prevalent one at an AF of 3.1%. Of the six reversion mutation clones detected after progression, one had previously been detected as the most prevalent clone among five different clones at week 61, and one was the originally detected first reversion mutation clone, albeit at a higher AF. The genomic, radiographic, and PSA dynamics are shown in Figure 4B.

4. Discussion

A known mechanism of acquired resistance to PARP inhibition in BRCA mutant cancers is secondary BRCA mutations that restore the ORF, protein function, and homologous repair capability. The investigation of secondary BRCA mutations presented here includes 100 TRITON2 [1] patients with mCRPC and a BRCA alteration treated with the PARPi rucaparib. Baseline BRCA genomics, such as mutations by gene (*BRCA1* or *BRCA2*), germline/somatic status, and alteration type, were comparable with what has been reported in this patient population [4,6]. Importantly, patients had not received prior platinum-based chemotherapy or PARPi, and no baseline BRCA reversion mutations were observed in any patients prior to treatment, implying that detectable BRCA reversion mutations developed under therapeutic pressure during rucaparib treatment, although pre-existing and emerging BRCA mutations below the assay limit of detection may have been missed.

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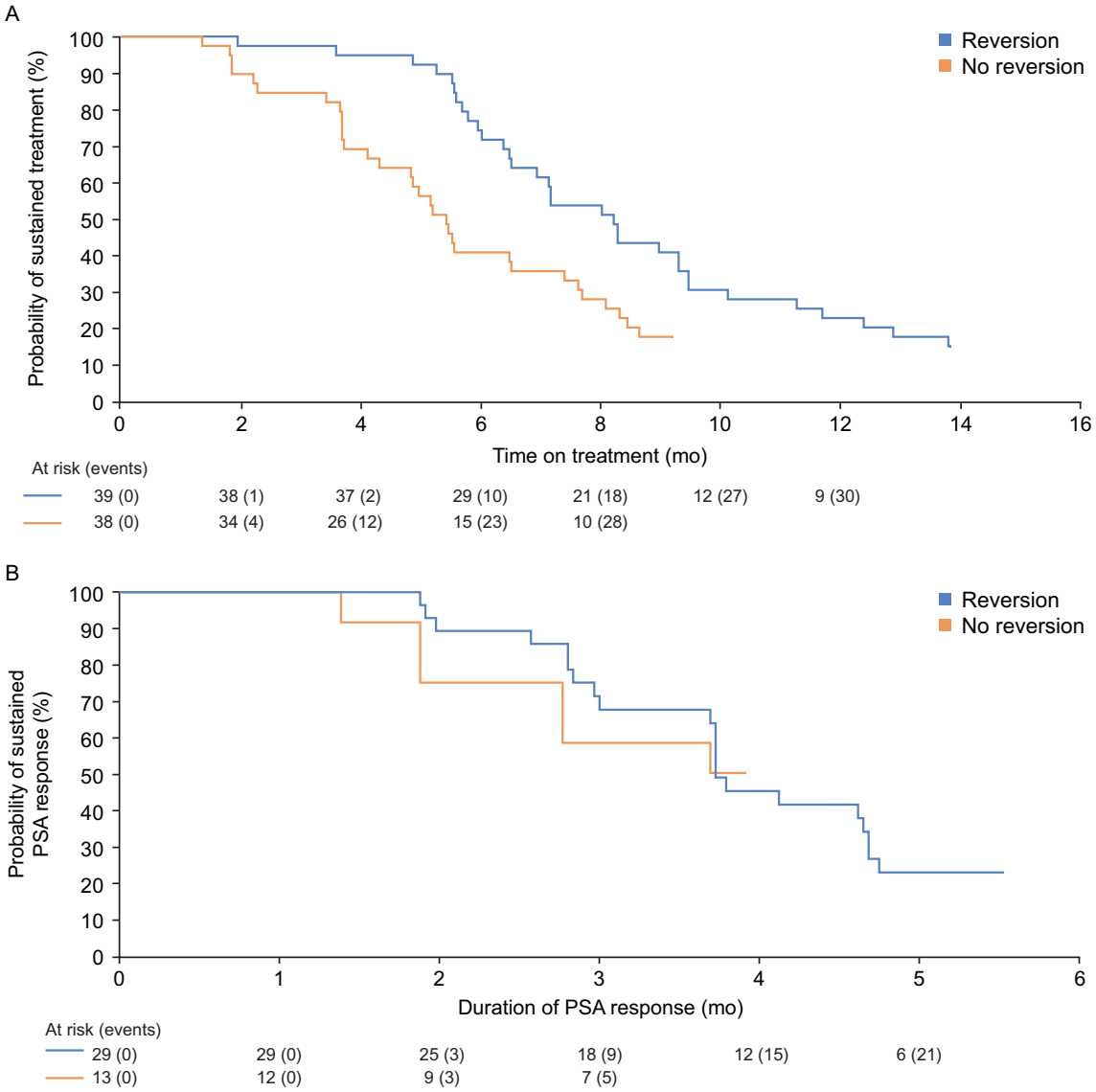

Fig. 3 – Association between detectable reversion mutations and patient clinical outcomes. (A) Time on treatment. (B) Duration of PSA response. PSA = prostate-specific antigen.

At EOT after progression on rucaparib, reversion mutations were detected in at least 39% of all patients with BRCA+ mCRPC. Rare cases of reversions to the original wild-type sequence [31] could not be detected by the assay used, and lower ctDNA fractions were found in samples without observed reversion mutations at progression, suggesting that additional cases of subclonal mutations may have been present but were not detected during testing. This possibility is raised by a recent report of an autopsy case where a multitude of subclonal mutations was detected in tumor tissue, but none were found via concurrent cfDNA testing [22]. These findings show that a high ctDNA fraction in patients' plasma creates a favorable condition for the detectability of secondary BRCA mutations, and their prevalence in 39% of patients reported here should be regarded as a lower limit.

The high rate of reversion mutations is remarkable compared with what has been observed in other cancer types [12], in particular, among cancers that are commonly treated with several lines of DNA damaging agents, such as platinum-based chemotherapy. For example, in studies of ovarian cancer, roughly 10% of patients are reported to have had reversion mutations before and an additional 10% after PARPi treatment [11], while in breast cancer such mutations have been reported in around 30–40% of patients undergoing PARPi treatment [13,32].

We hypothesize that prostate cancer is highly dependent on BRCA loss of function as a driver event (evidenced in part by the fact that roughly half of all BRCA alterations in mCRPC [33] are somatic compared with 18–30% in ovarian cancer [34–36]) and therefore more likely to be affected by resistance mechanisms that directly impact BRCA function, such as reversion mutations that allow the cancer cells to adapt to continued PARP inhibition. However, more comprehensive studies will be required to fully elucidate the underlying biological and genomic determinants for this difference.

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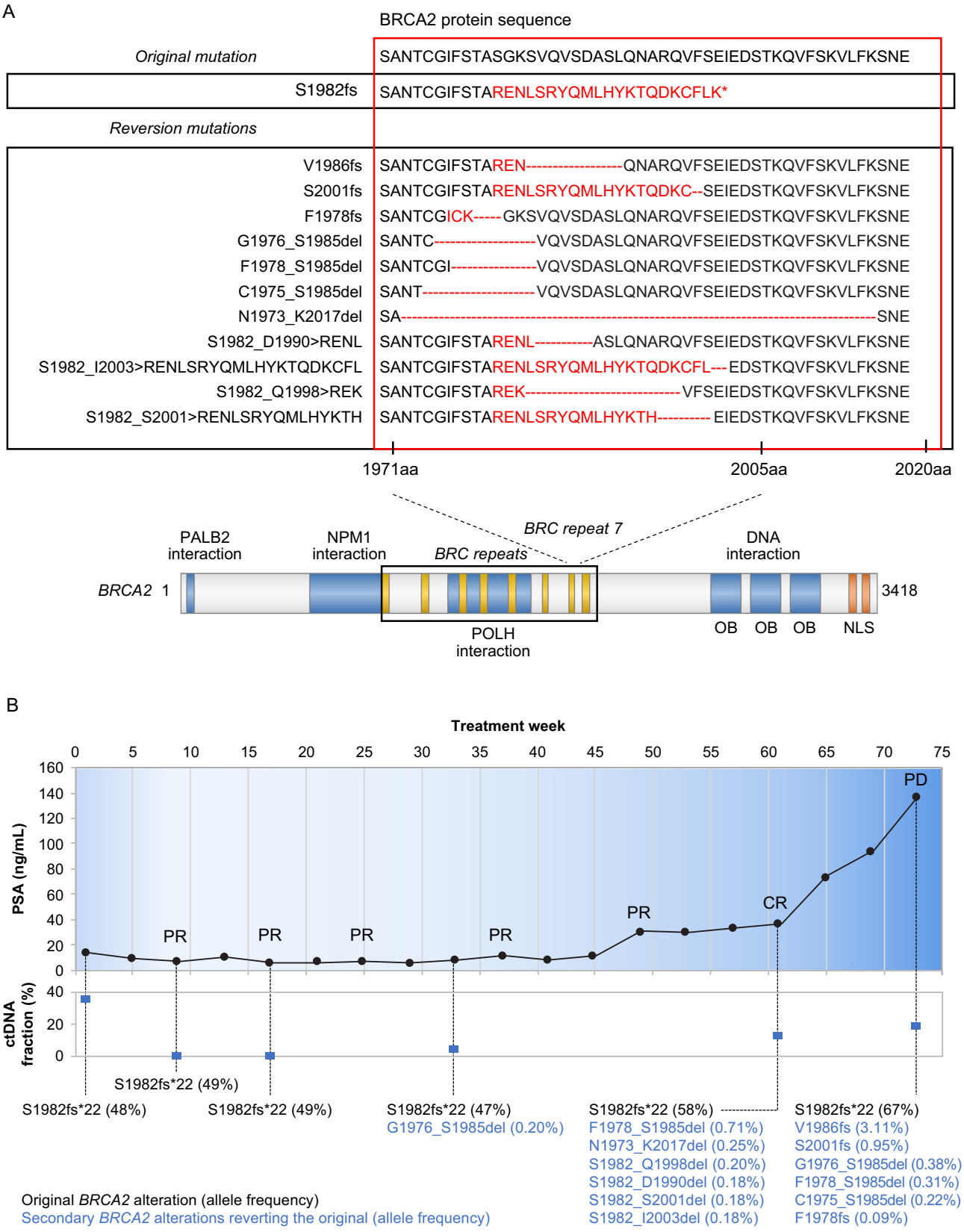

Fig. 4 – Genomics and course of clinical disease in a patient with BRCA+ mCRPC who progressed with multiple subclonal *BRCA2* reversion mutations. (A) An overview of all reversion mutation clones detected over time. (B) Dynamics of emergent *BRCA2* reversion mutations, radiographic response, and PSA levels. CR = complete response; ctDNA = circulating tumor DNA; mCRPC = metastatic castration-resistant prostate cancer; PD = progressive disease; PR = partial response; PSA = prostate-specific antigen.

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Patients developed reversion mutations at similar rates across both *BRCA* genes and irrespective of whether the initial BRCA mutations were germline or somatic. There was no preferred location of reverted mutations along the *BRCA* genes, and previously reported evidence for hotspots at the *BRCA2* N terminal or desert at the C terminus [31] could not be confirmed in this smaller data set. Additionally, there were no potentially confounding differences in baseline demographic characteristics between patients with and without reversions, including prognostic ones. Demographic or BRCA genomic characteristics that could serve as markers of patients' prognosis for developing reversion mutations were not identified.

Most patients had developed two or more secondary reversion mutations with AFs much lower than those of the original BRCA alterations. Combined with the observed absence of a dominant subclone in the vast majority of samples, this suggests that in most patients, multiple divergent subclones developed, driving the emerging PARPi resistance in parallel.

Rucaparib was the first DNA-damaging treatment administered to this cohort of patients with BRCA+ mCRPC, and the emergence of acquired resistance through reversion mutations in patients with reversible BRCA alteration types appeared to require sustained therapeutic pressure: patients with detectable reversion mutations at EOT were on treatment for a longer duration than patients without detectable reversion mutations, and nonresponders to therapy had lower rates of reversion mutations at EOT. These findings suggest that other mechanisms of resistance are involved or that the BRCA alteration was not a key oncogenic driver event in these cases.

5. Conclusions

The analyses presented here are the most comprehensive results examining BRCA reversion events in cfDNA from a prospective study incorporating somatic and germline testing as well as clinical outcome data. However, the analysis is limited to the detection of reversion mutations after progression, and it does not take into account the timing of onset of these reversions. An illustrative case study shown here demonstrates that clinical progression may not occur for some time after the emergence of a reversion mutation, suggesting that the appearance of a mutation should not be used for clinical decision-making. Future studies of the onset of BRCA reversion mutations and their relation to clinical benefit are needed to provide insights into the actionability of reversion mutations and develop effective therapies for resistant disease.

***Author contributions*:** Wassim Abida had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Loehr, Abida, Simmons, Nguyen.
Acquisition of data: Loehr, Abida, King, Hussain, Patnaik, Bryce, Castellano, Font, Shapiro, Zhang, Sautois, Vogelzang, Chatta, Courtney, Harzstark, Ricci, Watkins, Chowdhury.
Analysis and interpretation of data: Loehr, Despain, Abida, Simmons, Nguyen.
Drafting of the manuscript: Loehr, Abida, Despain, Simmons, Nguyen.
Critical revision of the manuscript for important intellectual content: Loehr, Abida, Simmons, Nguyen, Watkins.
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