Pancreatology 24 (2024) 1066–1072

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

A pilot study of chlorambucil in pre-treated metastatic pancreatic adenocarcinoma patients bearing germline BRCA or other DNA damage repair system variants

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ARTICLE INFO

Article history:
Received 16 April 2024
Received in revised form
27 August 2024
Accepted 8 September 2024
Available online 12 September 2024

Keywords:
BRCA
DNA damage repair system
Pancreatic ductal adenocarcinoma
Salvage therapy

ABSTRACT

Backgorund: Pancreatic adenocarcinoma remains a malignancy with a grim prognosis and scarce personalized treatment options. Pathogenic variants of DNA damage repair (DDR) genes are emerging as molecular targets, as they confer a higher sensitivity to DNA-damaging agents. This study aimed at assessing the activity of chlorambucil as salvage therapy in metastatic pancreatic cancer patients bearing a germline pathogenetic variant or variant of uncertain significance on a DDR-related gene.
Methods: Platinum-pretreated metastatic pancreatic cancer patients harbouring a germline variant on a DDR gene received chlorambucil at a daily oral dose of 6 mg/m^2 for 42 every 56 days for the first cycle and for 14 every 28 days for the following cycles, until disease progression or unacceptable toxicity. The primary endpoint was 6-month progression-free survival rate (PFS-6). Median progression-free survival (PFS) and overall survival (OS) were secondarily described.
Results: Twenty patients were enrolled between December 2020 and September 2022. PFS-6 was 5%, median PFS and OS were 1.6 months and 3.0 months, respectively. Grade-3 adverse events were observed in 25% of patients, while no Grade-4 toxicity was reported.
Conclusions: Single agent chlorambucil did not show sufficient signal of activity to warrant its further investigation in metastatic pancreatic cancer patients bearing a DDR-related germline alteration.
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1. Introduction

Pancreatic cancer (PC) is the sixth leading cause of cancer-related deaths worldwide, with a mortality-to-incidence ratio that has remained almost unchanged over the past decades [1,2]. The current standard first-line chemotherapy for metastatic disease

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https://doi.org/10.1016/j.pan.2024.09.006
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consists of single or multi-agent cytotoxic regimens (FOLFIRINOX or gemcitabine and nab-paclitaxel) [3,4]. Despite the growing number of clinical trials assessing the efficacy of different second-line treatments, the optimal strategy in this setting remains unclear [5–7].

The recent introduction of germline and somatic testing of PC patients has revealed a complex mutational landscape underlying this cancer type that might pave the way for better patient selection for current treatment options and for the discovery of novel therapeutics [8]. In particular, landmark genome-wide studies shed light to the existence of a distinct subtype of PC with high genomic instability that is related to a mutational signature of DNA damage repair (DDR) deficiency [8,9]. Among the most frequent DDR germline pathogenic variants (gPV) observed in PC, those impairing BRCA1, BRCA2, PALB2, ATM and CHEK2 lead to a homologous recombination repair deficient (HRD) phenotype [10–13]. Moreover, variants of uncertain significance (VUS) on DDR genes are often identified and even though their role in PC needs to be clarified, they might correlate with worse outcomes [14,15].

Although defects in DDR system are causative of carcinogenesis and tumor progression, they also confer exquisite sensitivity to DNA damage-inducing drugs, especially those that interfere with DNA replication by inflicting DNA cross-links (i.e. platinum compounds and DNA alkylators) [13,16–19]. In line, the HRD signature seems to be predictive of response to platinum agents and poly-adenosine diphosphate-ribose polymerase (PARP) inhibitors such as Olaparib, with documented improvement in clinical outcomes [20]. Despite an initial good response to these agents, most patients face disease progression [21]. In order to overcome resistance while exploiting the impaired DDR, different DNA-damaging drugs, such as alkylating agents, might represent a possible therapeutic option [22]. Among these, chlorambucil, a nitrogen mustard that has been historically used for the treatment of hematological malignancies such as chronic lymphocytic leukaemia and B-cell lymphomas, has shown toxicity against BRCA1/2 deficient human cells and xenograft tumors [23–25]. Although similar to cisplatin in forming DNA cross-links, evidence suggests that chlorambucil may counteract platinum and Olaparib resistance while exposing normal cells and tissues to a lower toxicity [23,26].

Based on these considerations, we conducted a phase 2 trial, PACT-29 SALE (Pancreatic AdenoCarcinoma Trial-29 Salvage Leukeran; ClinicalTrials.gov Identifier: NCT04692740; EudraCT number: 2020-002949-42), to explore the activity of chlorambucil in metastatic PC patients bearing a gPV or VUS on a DDR gene and that had previously received at least one platinum-based chemotherapy.

2. Materials and methods

This pilot trial was designed as a mono-institutional, prospective, single-arm phase II study.

2.1. Patients

Patients aged ≥18 years, with pathologically confirmed metastatic pancreatic adenocarcinoma, an HRD-related gene VUS or gPV, a Karnofsky performance status (KPS) ≥60, and measurable disease according to RECIST criteria [27] were eligible for the trial. Previous treatment with platinum salt-containing regimen was mandatory. Other prior chemotherapies, including maintenance treatment with PARP-inhibitors, were allowed as long as they had been discontinued from at least 2 weeks. Adequate bone marrow, renal, liver, and cardiac functions were required. Patients were categorized into 3 cohorts: cohort A included patients with a gPV on BRCA1 or BRCA2; cohort B included patients with a variant of uncertain/unknown significance (VUS) on BRCA1 or BRCA2; cohort C included patients with a gPV and/or VUS of other genes associated with HRD.

Patients with severe comorbidities, history of prior or concurrent malignancy at other sites except for surgically cured in situ carcinoma, non-melanoma resected skin cancer and other malignancies showing no evidence of disease from at least 5 years, or receiving a concurrent treatment with other experimental drugs, were deemed ineligible.

2.2. Outcomes

The primary outcome measure was the rate of patients who were progression-free at 6 months from treatment start (PFS-6).

Secondary endpoints included overall survival (OS), progression-free survival (PFS), objective response rate (ORR), serum carbohydrate antigen 19.9 (CA19.9) response rate, and safety profile. OS was defined as the time between the start of treatment and the date of death from any cause or, when unknown, the last date the patient was known to be alive. PFS was defined as the time between the date of treatment beginning and the date of documented radiological disease progression or death, whichever occurred first; patients without an event (disease progression or death) were censored on the last follow-up date. ORR was defined as the incidence of either complete or partial responses on CT scans according to RECIST criteria version 1.1 [27]; subjects prematurely discontinuing the investigational drug in the absence of a post-baseline tumor response assessment or with an unconfirmed complete or partial response were considered as non-responders. CA19.9 response rate was defined as CA19.9 percentage variation at nadir (minor value assessed while on treatment) with respect to basal value, only in patients with elevated (namely over superior normal laboratory level) basal CA19.9 level. With regards to CA19.9 variation, patients were classified as non-responders (<50%), minor responders (between 50% and 89%) or major responders (≥90%) [28].

All efficacy and safety analyses were conducted in the intent-to-treat population. Safety was defined according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 5.0 (NCI-CTC) [29]. Moreover, an analysis of chlorambucil dose intensity was performed and reported as the percentage of the ratio between the delivered average dose and the intended average dose per each cycle.

All patients provided written informed consent. The trial was approved by the local ethics committee (San Raffaele Hospital, Milan, Italy; institutional ethical clearance reference number: 117/INT/2020, September 16, 2020) and was conducted with the principles of good clinical trial practice according to the Declaration of Helsinki.

2.3. Treatment schedule and dose adjustment

Chlorambucil was administered per os at a dose of 6 mg/m^2 daily for 42 consecutive days (weeks 1–6) for the first cycle that lasted 56 days (8 weeks). After restaging, responder patients (complete or partial response) and those with stable disease (SD) received the following cycles at a dose of 6 mg/m^2 daily for 14 consecutive days every 28 days unless there was radiologic evidence of disease progression according to RECIST criteria [27] or unacceptable toxicity based on the clinical judgment or patient/physician decision otherwise. Supportive care could be

Table 1
Patients' characteristics.

Patients	All patients N = 20	BRCA1-2 gPV N = 9	BRCA1-2 VUS N = 6	DDR-gene variant N = 5
	N (%)	N (%)	N (%)	N (%)
Age (years)				
Median (range)	59.5 (38–74)	55.0 (38–74)	64.0 (43–73)	58.0 (55–66)
Gender				
Male	9 (45)	5 (56)	2 (33)	2 (40)
Female	11 (55)	4 (44)	4 (67)	3 (60)
BMI[a]				
≤18.5	3 (15)	1 (11)	2 (33)	0
18.6–25	10 (50)	5 (56)	2 (33)	3 (60)
25.1–30	3 (15)	1 (11)	1 (17)	1 (20)
>30	4 (20)	2 (22)	1 (17)	1 (20)
KPS[b]				
80-100	18 (90)	8 (89)	6 (100)	4 (80)
60-70	2 (10)	1 (11)	0	1 (20)
Comorbidity[c]				
Yes	14 (70)	7 (78)	5 (83)	2 (40)
No	6 (30)	2 (22)	1 (17)	3 (60)
Previous cancer				
Yes	4 (20)	3 (33)	1 (17)	0
No	16 (80)	6 (67)	5 (83)	5 (100)
Tumor characteristics				
Number of lesions				
2-5	3 (15)	3 (33)	0	0
>5	14 (70)	4 (44)	5 (83)	5 (100)
NA[d]	3 (15)	2 (22)	1 (17)	0
Number of metastatic sites				
1	3 (15)	2 (22)	0	1 (20)
2	6 (30)	2 (22)	3 (50)	1 (20)
>2	8 (40)	3 (33)	2 (33)	3 (60)
NA[d]	3 (15)	2 (22)	1 (17)	0
Liver metastasis				
Yes	15 (75)	7 (78)	4 (67)	4 (80)
No	4 (20)	1 (11)	2 (33)	1 (20)
NA[d]	1 (5)	1 (11)	0	0
Median CA 19.9 (UI)				
Median (range)	3784 (0.8–114800)	4495 (10–114800)	502 (19–10000)	34594 (0.8–68000)
>ULN[e]	15 (75)	7 (78)	4 (67)	4 (80)
<ULN[e]	5 (25)	2 (22)	2 (33)	1 (20)
NLR[f]				
Median (range)	3.2 (0.5–13.5)	3.6 (0.5–5.2)	2.9 (0.6–5.9)	3.1 (1.9–13.5)

[a] Body mass index.
[b] Karnofsky performance status.
[c] Diabetes and/or hypertension.
[d] Not available.
[e] ULN: upper limit of normality.
[f] Neutrophil-to-lymphocyte ratio at baseline.

administered according to the investigator's discretion.

A dose reduction of 33% was applied in case of any grade ≥3 toxicity. Whenever a second episode of grade ≥3 toxicity occurred, chlorambucil was suspended until grade 1 recovery and administered with the same dose reduction for 10 days only. Drug administration was delayed by 1 week in the event of uncomplete recovery.

2.4. Study evaluations

Complete blood count, a comprehensive biochemical panel including liver and kidney function and circulating tumor markers CA19.9 and/or carcinoembryonic antigen (CEA) were performed within 7 days before the beginning of the therapy. During chemotherapy, blood count was repeated every week and a biochemical panel every 3 weeks or as clinically indicated. Tumor markers were measured before each cycle and every 8 weeks until progression.

A tumor radiological assessment with contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) was performed at screening and every 8 weeks thereafter until disease progression.

2.5. Statistical analysis

At the time of the trial design, it was not possible to precisely determine the sample size due to the rarity of the target population and the lack of benchmark data. Assuming that the PFS-6 rate for a previously treated PC patient is approximately 20% [20], we planned to enroll 30 patients, 10 for each cohort (namely A, B and C), defined by a different type of molecular alteration. Considering the low frequency of DDR genes variants in PC patients, we considered that we would have needed 30 months to complete the enrollment and 36 months to complete the study. If at least 3 patients per cohort were PFS-6, a larger phase II trial would have been conducted.

Survival distribution was estimated using the Kaplan–Meier method; medians and two-sided 95% CIs were provided. AEs and SAEs were graded for intensity according to the NCI-CTC version 5.0

Table 2
Details of previous treatments.

Variable	All patients N = 20	BRCA1-2 gPV N = 9	BRCA1-2 VUS N = 6	DDR-gene variant N = 5
	N (%)	N (%)	N (%)	N (%)
Number of prior chemotherapy lines				
Median (range)	3 (1–5)	2 (1–5)	2.5 (2–3)	3 (2–4)
≤2	10 (50)	5 (56)	3 (50)	2 (40)
3	6 (30)	2 (22)	3 (50)	1 (20)
≥4	4 (20)	2 (22)	0	2 (40)
Surgery[a]				
Yes	10 (50)	5 (56)	2 (33)	3 (60)
No	10 (50)	4 (44)	4 (67)	2 (40)
Prior platinum-based chemotherapy				
1st line				
Yes	15 (75)	8 (89)	4 (67)	3 (60)
No	5 (25)	1 (11)	2 (33)	2 (40)
Beyond 1st line				
Yes	9 (45)	4 (44)	2 (33)	3 (60)
No	11 (55)	5 (56)	4 (67)	2 (40)
Rechallenge				
Yes	8 (40)	5 (56)	1 (17)	2 (40)
No	12 (60)	4 (44)	5 (83)	3 (60)
Months to platinum failure[b]				
Median (range)	9.2 (2.3–80.4)	13.4 (10.1–80.4)	9.6 (2.3–16.3)	5.8 (2.8–10.8)
<6	6 (30)	1 (11)	2 (33)	3 (60)
6-12	7 (35)	3 (33)	2 (33)	2 (40)
>12	7 (35)	5 (56)	2 (33)	0
Olaparib maintenance				
Yes	8 (40)	6 (67)	0	2 (40%)
No	12 (60)	3 (33)	6 (100%)	3 (60%)
Months to Olaparib failure[c]				
<6	2 (25)	2 (33)	NA[d]	NA[d]
6-12	3 (37)	3 (50)	NA[d]	NA[d]
Uknown	3 (37)	1 (17)	NA[d]	2 (100)

[a] Surgical resection of primary tumor.
[b] Calculated from the first platinum-based CHT start to the evidence of PD.
[c] Calculated from the Olaparib maintenance treatment start to the evidence of PD.
[d] NA: not applicable.

[29] and presented by summarizing the number and percentage of patients reporting one. All statistical analyses were conducted using MedCalc® version 22.018.

3. Results

3.1. Patients

Between December 2020 and September 2022, 20 patients with metastatic pancreatic adenocarcinoma and a germline variant on a DDR-related gene were enrolled in the trial. Cohort A included 9 patients harboring a gPV on BRCA2; cohort B included 6 patients with a VUS on BRCA1 or BRCA2; cohort C included 5 patients with a non-BRCA DDR-related gene variant. In this latest cohort, one eligible individual carried a gPV on a non-BRCA DDR-gene (PALB2), while the other 4 patients had a VUS on a DDR-related gene. Tables 1 and 2 summarize patients' clinical characteristics at baseline and their previous treatments, respectively.

3.2. Treatment

The median treatment duration for all patients was 42 days (range 10–210 days), with a median relative dose intensity of 85.0% (IQ range from 49.4% to 94.6%) for all cohorts, in particular median dose intensity was 88.7%, 76.9% and 65.4% for cohort A, B, and C, respectively. The median treatment cycle number for all patients was 1 (range 1–7). Dose reduction occurred for a total of 9 times in 8 patients (40%) due to concurrent infections (4), hematological toxicity (1), abdominal pain (1), patient's mistake (1) and worsening of the general conditions (2).

3.3. Outcomes

All patients included in the study faced Progressive Disease (PD) during the treatment with chlorambucil and only one patient in cohort A out of the 20 totally treated (5%) was PFS-6. For patients included in cohort A, mPFS was 1.6 months (range 0.7–7.0). In consideration of their well-known response to platinum-based chemotherapies and of the available pre-clinical data [23,30], we considered patients with a BRCA1/2 gPV to have the highest probability of being susceptible to chlorambucil. Given that only one patient in cohort A reached PFS-6, we deemed it less likely to achieve the primary endpoint in the other two cohorts and halted the study prematurely. A total of 6 patients were included in cohort B and obtained a mPFS of 1.6 (range 0.6–3.0). Finally, patients in cohort C showed a mPFS of 1.4 (range 0.3–2.6). Median OS was 4.2 (2.7–14.3), 2.6 (2.2–7.6) and 2.0 (0.6–3.9) for cohort A, B, and C, respectively (Fig. 1). Taken all subgroups together, mOS was 3.0 months (0.6–14.3). At least 3 patients in cohort A and no patient in the other cohorts received post-progression treatment.

No complete or partial response was observed; two patients in cohort A and one patient in cohort C obtained SD as best response; the other 17 patients had PD as best response at first evaluation (85%). Out of the 15 patients expressing CA19.9 at baseline, no major biochemical response was observed and only one patient in cohort A showed a minor biochemical response (reduction of serum CA19.9 by 62.4%).

Treatment-related mild or moderate adverse events were

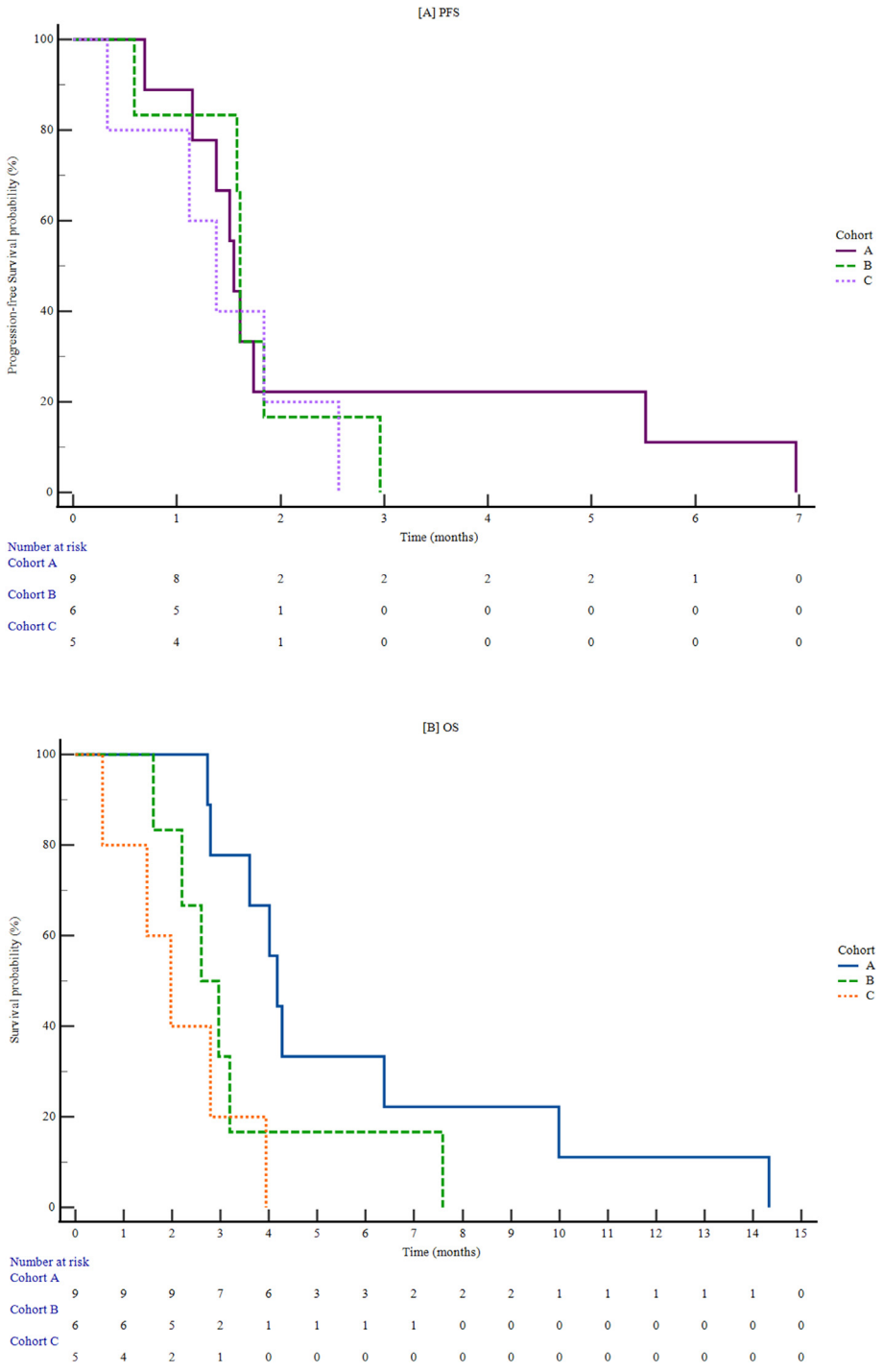

Fig. 1. Kaplan-Meier curves of PFS (A) and OS (B) divided by cohort.

reported in 13 patients (65%); 3 patients (15%) developed severe anemia and two patients reported Grade 3 fatigue (10%). No life-threatening adverse event was observed (Table 3).

4. Discussion

This single institution phase II trial aimed to assess the activity of chlorambucil as salvage therapy in pre-treated patients with metastatic pancreatic adenocarcinoma bearing a germline variant on BRCA1/2 or other DDR genes. As prespecified in the protocol, enrollment was stopped because of an insufficient PFS-6 among patients included in cohort A (1 out of 9). The primary endpoint was not met and PFS-6 for all patients was 5%. Neither objective radiological response nor major biochemical response was observed although a stable disease $\geq$8 weeks was seen in 15% of patients.

Although it is not possible to directly compare the responses we have obtained with those expected with the use of other agents, the survival outcomes of the BRCA gPV subgroup seem to be consistent with those reported in a phase 2 trial evaluating the efficacy of

Table 3
Details of adverse events.

Adverse event	All patients N = 20	BRCA1-2 gPV N = 9	BRCA1-2 VUS N = 6	DDR-gene variant N = 5
	N (%)	N (%)	N (%)	N (%)
Anemia				
Grade 0	16 (80)	8 (89)	4 (67)	4 (80)
Grade 1	1 (5)	0	1 (17)	0
Grade 2	0	0	0	0
Grade 3	3 (15)	1 (11)	1 (17)	1 (20)
Grade 4	0	0	0	0
Anorexia				
Grade 0	17 (85)	8 (89)	4 (67)	5 (100)
Grade 1	2 (10)	1 (11)	1 (17)	0
Grade 2	1 (5)	0	1 (17)	0
Grade 3	0	0	0	0
Grade 4	0	0	0	0
AST/ALT[a] elevation				
Grade 0	19 (95)	8 (89)	6 (100)	5 (100)
Grade 1	1 (5)	1 (11)	0	0
Grade 2	0	0	0	0
Grade 3	0	0	0	0
Grade 4	0	0	0	0
Constipation				
Grade 0	17 (85)	8 (89)	5 (83)	4 (80)
Grade 1	2 (10)	0	1 (17)	1 (20)
Grade 2	1 (5)	1 (11)	0	0
Grade 3	0	0	0	0
Grade 4	0	0	0	0
Fatigue				
Grade 0	13 (65)	7 (78)	3 (50)	3 (60)
Grade 1	2 (10)	1 (11)	0	1 (20)
Grade 2	3 (15)	0	2 (33)	1 (20)
Grade 3	2 (10)	1 (11)	1 (17)	0
Grade 4	0	0	0	0
Fever				
Grade 0	17 (85)	7 (78)	6 (100)	4 (80)
Grade 1	2 (10)	1 (11)	0	1 (20)
Grade 2	1 (5)	1 (11)	0	0
Grade 3	0	0	0	0
Grade 4	0	0	0	0
Infection				
Grade 0	18 (90)	9 (100)	6 (100)	3 (60)
Grade 1	2 (10)	0	0	2 (40)
Grade 2	0	0	0	0
Grade 3	0	0	0	0
Grade 4	0	0	0	0
Nausea				
Grade 0	19 (95)	9 (100)	5 (83)	5 (100)
Grade 1	1 (5)	0	1 (17)	0
Grade 2	0	0	0	0
Grade 3	0	0	0	0
Grade 4	0	0	0	0
Platelet count decreased				
Grade 0	18 (90)	8 (89)	6 (100)	4 (80)
Grade 1	1 (5)	1 (11)	0	0
Grade 2	1 (5)	0	0	1 (20)
Grade 3	0	0	0	0
Grade 4	0	0	0	0

[a] AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

second line Veliparib in patients bearing a BRCA gPV (mPFS of 1.5 versus 1.7 months and mOS of 4.2 versus 3.1 months with chlorambucil and Veliparib, respectively) [31]. Similar survival figures were observed in the subgroup of non-BRCA gPV PC population enrolled in the KEYNOTE-158 trial, that investigated the role of the anti-PD1 monoclonal antibody pembrolizumab in non-colorectal cancer patients with mismatch repair deficiency, showing a mPFS of 2.1 months and a mOS of 3.7 months [32]. These results appear unsatisfactory when compared to those reported by Kaufman et al. with the administration of Olaparib monotherapy to 23 pre-treated PC patients bearing a gPV on BRCA1/2 (mPFS of 4.6 months and mOS of 9.8 months) [33]. Of note, although only 65% of PC patients included in this study had received prior platinum-based chemotherapies, no difference in response rates was observed when compared to the platinum-naive counterpart [33]. However, the small number of PC patients included in the abovementioned study hampers drawing firm conclusions on possible differences of response among the two subgroups.

In our population, we observed a sign of better prognosis in the BRCA gPV patients, that showed a similar PFS yet a longer OS than the other subgroups. After 4-months, 55% of patients harboring a BRCA gPV and no patient with a VUS was alive. This result may be due to the fact that BRCA gPV patients had a smaller proportion of lesions and of metastatic sites at the time of enrolment and were on average in better clinical conditions during the treatment period, which is consistent with the finding that they have received a higher median dose-intensity than the other subgroups. Moreover, at least 3 of the BRCA-mutated patients (33%) and no patient in the other cohorts received a further line of treatment at progression, which might have impacted the final OS result. Interestingly, although the prognostic value of BRCA1/2 gPVs in PC is currently unknown, these variants seem to be associated with an earlier onset of PC, a more aggressive disease and with a higher stage at diagnosis [34]. When not exposed to platinum-agents, gBRCA1/2-related PCs tend to show worse survival outcomes compared to their wild-type counterparts [14]. However, this difference seems to revert when mutated patients are exposed to platinum-based chemotherapies [21]. In addition, no study has evaluated the efficacy of second line treatments on PC patients bearing a VUS on a DDR-related gene so far. One possible explanation might be that patients with a BRCA1/2 VUS seem to be more similar to wild-type PC patients than to those with a BRCA1/2 gPV [15,35], therefore their response to treatments and their prognosis are comparable to the ones expected in the general population.

Taken all subgroups together, patients included in this pilot trial did not benefit from treatment with chlorambucil, obtaining a mPFS of 1.6 months and a mOS of 3.0 months. In contrast, other studies investigating chemotherapy regimens in second-line PC have reported inconsistent but apparently more favorable mPFS and numerically longer mOS, that ranged from 1.5 to 5.1 months and from 3.3 to 9.9 months, respectively [35]. Although these results appear slightly better than the ones we have observed, a proper comparison cannot be made due to different selection criteria and patients' characteristics. In particular, most patients in former studies had received only one previous line of therapy with gemcitabine while 50% of our patients had received at least 3 prior lines of treatment [5,6], hence their tumor was probably less susceptible to a further chemotherapy for a likely acquired resistance. Moreover, our patients presented a more unfavorable disease burden at the time of enrollment, with 40% of individuals having $\geq$3 metastatic sites. In contrast, most patients included in NAPOLI-1 trial had <2 metastatic sites, while PANCREOX and CONKO-003 studies included a proportion of patients with M0 disease (around 6.5% and 11.8%, respectively) [5–7]. Additionally, OS differences may be attributable not only to treatment efficacy, but also to the unbalanced proportion of patients receiving post-progression therapies among studies.

Overall, despite the analysis being limited by the small number of patients and by the lack of a comparison arm, no clear benefit of the use of chlorambucil in pre-treated metastatic PC was identified in this study. One of the possible reasons for its inefficacy might be that, similarly to ovarian cancer cells, platinum-exposed BRCA-mutated PC cells become unresponsive to DNA-damaging drugs after developing secondary BRCA mutations that result in the restoration of homologous recombination repair (HRR) [36,37]. Moreover, treatment might positively select BRCA wild-type cells that seem to exist at baseline as subclones of BRCA-mutated

cancers and such subclonality may lead to the accumulation of platinum/PARP-inhibitors unresponsive wild-type cells during treatments [38]. Since all patients were heavily pre-treated with DDR-targeting regimens, it can be hypothesized that chlorambucil failure was caused by a previously acquired resistance and that the molecular consequences of such a selective pressure might negatively impact the response to any further approach targeting DDR pathways. To date, the exact underlying mechanisms remain unclear and further studies are required to unveil them. Despite this hypothesis, no study has investigated chlorambucil's role either in the first line nor in the maintenance setting and no evidence of its efficacy regardless of resistance has been provided in solid tumors so far. Therefore, we cannot exclude that PC patients are unresponsive to chlorambucil regardless of their acquired chemotherapy resistance, mutational status and disease burden.

In conclusion, although chlorambucil was well tolerated, we did not see a sufficient signal of activity to warrant further investigation of a monotherapy with this alkylating agent in heavily pre-treated metastatic PC patients. DNA repair alterations remain the most frequent druggable mutations in PC and various ongoing trials are investigating the use of targeted treatments with PARP-inhibitors in second (or more advanced) line setting in PC patients bearing a gPV on a DDR-gene (NCT02677038; NCT01078662; NCT03601923), even in combination with immunotherapy (NCT04493060). Future studies are needed to better understand the molecular biology of DDR-mutated PC and to optimize treatment sequence in these patients in order to obtain better outcomes and to limit resistance to therapy.

Acknowledgments

This work was supported by Associazione Italiana per la Ricerca sul Cancro (AIRC under 5 per Mille 2019 – ID. 22737 program – P.I. MC Bonini, Group leader, M Reni).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2024.09.006.

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