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A phase II study of Navitoclax (ABT-263) as single agent in women heavily pretreated for recurrent epithelial ovarian cancer: The MONAVI – GINECO study

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HIGHLIGHTS

- Navitoclax in monotherapy has poor activity with acceptable tolerance in heavily pretreated ovarian cancer patients.
- The 3-month progression-free survival rate was 22.7% [95% CI: 13.2;39.2].
- Thrombocytopenia was the major expected reversible side effect, unrelated to clinically significant bleeding events.
- BIM expression, alone or combined with Mcl-1 and/or p-ERK, was not found to be predictive of Navitoclax benefit.

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ABSTRACT

Background. There are limited treatment options for ovarian cancer patients with early relapse after platinum chemotherapy. In preclinical studies, we previously demonstrated the promising activity of ABT-737, a Bcl-2/Bcl-x_L anti-apoptotic protein inhibitor, in chemo-resistant ovarian cancer cells and tumors, suggesting its potential activity in platinum-resistant patients.

Methods. We conducted a prospective multicenter single-arm phase II study to assess the efficacy of Navitoclax (orally available ABT-737 analogue) monotherapy in 46 heavily pretreated (2–12 lines, median = 4) patients with high-grade serous platinum-resistant ovarian tumors. Navitoclax was administered at the daily dose of 150 mg during a lead-in period (7–14 days) and then increased to 250 mg daily in the absence of dose-limiting thrombocytopenia (<G3). Progression-free survival (PFS) based on RECIST v1.1 criteria was the primary endpoint. Analysis of efficacy according to the expression of Bcl-2 family proteins in tumor biopsies was also planned.

Results. The 3-month PFS was 22.7% [$_{95\%}$CI: 13.2–39.2], median PFS was 1.64 months [$_{95\%}$CI: 1.58–2.30]. There were 16 (35.6%, $_{95\%}$CI: 22.3–51.3) overall responses (RECIST v1.1): 1 partial response and 15 stable diseases. No correlation between the expression of Bim, Mcl-1 and P-ERK with clinical response was found in this study. Thrombocytopenia was the major side-effect (G3/4: $n = 12$; 26%), leading to pursue at the daily dose of 150 mg in 8 patients and to discontinue treatment in 3 patients. Neither significant bleeding nor toxic death were observed.

Conclusions. Navitoclax monotherapy had poor activity that was not correlated with the expression of Bim, Mcl-1 and P-ERK, without unacceptable toxicity.

Trial Registration: Clinicaltrials.gov identifier: NCT02591095

1. Introduction

Ovarian cancer is the leading cause of death from gynecological malignancies. The mainstay of ovarian cancer first line therapy includes cytoreductive surgery and platinum-taxane doublet chemotherapy, associated in most of cases with the anti-angiogenic bevacizumab drug [1,2]. Maintenance treatment, based on bevacizumab and/or a PARP (poly(ADP-ribose) polymerase) inhibitors (niraparib, olaparib, and rucaparib) is nowadays part of the initial treatment [3–7]. However, after first line treatment, 80% of ovarian cancer patients experience recurrence that ultimately results in death as a result of the emergence of chemotherapy resistance, especially to platinums [8].

Therapeutic options for patients with platinum-resistant or previously heavily pretreated ovarian cancer are limited, and associated with poor prognosis. They are mainly based on cytotoxic agents, such as topotecan, pegylated liposomal doxorubicin (PLD), weekly paclitaxel, gemcitabine, cyclophosphamide or topotecan [9–12]. In this situation, adding bevacizumab to non-platinum monotherapy was shown to improve progression-free survival (PFS) and overall response rate [13], and is currently the only antiangiogenic agent approved in this setting. As for PARP inhibitors, they are not approved in platinum-resistant ovarian cancer, but are investigated alone or in combination in several trials. In addition, checkpoint inhibitors have failed to show activity in early relapse ovarian cancer [14].

In this context, the management of patients with platinum-resistant ovarian cancer remains a challenge and warrants to explore new drugs with different mechanisms of action from conventional chemotherapy or anti-angiogenic agents that could overcome or reverse platinum resistance. Targeting anti-apoptotic proteins of the Bcl-2 family thus appears as an interesting strategy [15–17]. Bcl-2 is a central apoptotic inhibitor whose overexpression is related not only to malignancy transformation but also to chemotherapy resistance [18,19]. In ovarian carcinoma, platinum resistance is associated with strong protection against apoptotic cell death, particularly due to the high expression level of the anti-apoptotic Bcl-x_L protein [20]. In vitro, the targeting of Bcl-x_L restores sensitivity of ovarian carcinoma cells to platinum compounds [20–23]. Moreover, we previously showed that Bcl-x_L and Mcl-1 cooperate to protect ovarian cancer cells from apoptosis and that their concomitant inhibition leads to massive apoptosis in the absence of chemotherapy, even in cells refractory to conventional chemotherapy [23]. The development of strategies able to inhibit these targets effectively in patients thus represents an attractive alternative to conventional therapeutics for the therapeutic care of chemo-resistant or heavily pretreated ovarian carcinomas. ABT-737 is a selective high-affinity small molecule that inhibits the anti-apoptotic proteins Bcl-2, Bcl-X_L, and Bcl-w. In a preclinical study [24], our team analyzed the response of 25 high-grade serous ovarian cancer (HGSOC) sliced samples from patients naïve of chemotherapy, exposed ex vivo to ABT-737 alone or in combination with carboplatin. In this condition, ABT-737 induced apoptotic cell death in 20–80% of cancer cells as a single agent in 14 of the 25 tumor samples and its efficacy was not significantly improved when combined with carboplatin. This observation underlines the interest of this molecule as a single agent for the treatment of ovarian cancers, thus avoiding combined toxicities. Interestingly, the response to ABT-737 was correlated with the high expression level of the BH3-only pro-apoptotic protein Bim, associated with a low expression of anti-apoptotic protein Mcl-1 (able to sequester Bim) and/or with a low level of P-ERK (a kinase able to phosphorylate Bim, leading to its inactivation and subsequent degradation by proteasome).

Navitoclax (ABT-263) is an orally bioavailable analog of ABT-737 [25,26]. It binds to the latter and releases apoptosis inducers (BH3-only and multidomain proteins) such as Bim, Bax or Bak. It has been assessed clinically in several monotherapy trials and in combination with chemotherapy in both solid tumors and hematologic malignancies. Effective tumor regression under Navitoclax treatment was shown in patients with small-cell lung cancer and acute lymphocytic leukemia, albeit with a decrease in platelet counts [27–31].

In this context, we implemented the French MONAVI-GINECO trial, a phase II study to assess the efficacy of Navitoclax as a single agent in women heavily pretreated for recurrent epithelial ovarian cancer.

2. Materials and methods

2.1. Study design

This is a French multicenter, prospective, single-arm, phase II trial assessing the activity of Navitoclax as single agent in heavily pretreated recurrent ovarian cancer patients. It was scientifically approved by and conducted in collaboration with the ARCAGY GINECO Intergroup (academic clinical research group specializing in gynecological oncology).

2.2. Study objectives

The primary objective was to determine the activity of Navitoclax in patients heavily pretreated for recurrent epithelial ovarian cancer, based on PFS as assessed by RECIST v1.1 criteria. Secondary objectives were to explore its activity in patients with a high Bim protein expression level as determined by immunohistochemistry, and to assess overall survival, objective response rate and toxicity profile of Navitoclax single-agent treatment. As an exploratory objective, tumor response was described for patients who were rechallenged with platinum-based chemotherapy after Navitoclax therapy.

2.3. Eligibility criteria

Patients with histologically and/or cytologically documented high-grade serous epithelial platinum-resistant or refractory cancer of the ovary, Fallopian tube or peritoneum, defined as relapsing within 6 months after a platinum-based chemotherapy or as progressing during a platinum-based chemotherapy (except refractory patients in first line) were eligible. Patients had to have received at least two prior lines of treatments, including at least one platinum-based regimen, whatever the line. Patients may have received other nonplatinum-based chemotherapy after the last platinum-based regimen. The number of prior lines of therapies was not limited. Patients were required to have documented disease progression with measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Biopsy of relapsed disease was mandatory in this study before initiation of Navitoclax treatment. Other eligibility criteria included performance status 0–2, LVEF >50%, absolute neutrophil count ≥1500/mm^3, platelets ≥150,000/mm^3, hemoglobin ≥9.0 g/dL, serum creatinine ≤1.2 mg/dL or calculated creatinine clearance ≥60 mL/min, AST/ALT ≤3.0-fold the upper limit of normal (ULN); [subjects with liver metastasis could have AST, ALP, and ALT less than or equal to 5.0-fold ULN], bilirubin ≤1.25-fold ULN, coagulation: aPTT and PT not to exceed 1.2-fold ULN.

The local ethics committee approved the study protocol (Ref. 2015–07, Comité de protection des personnes Nord-Ouest III). All patients gave written informed consent before any study procedure. The study is registered as EUDRACT 2015–00193-35, clinical trial NCT02591095.

2.4. Treatment schedule

The treatment schedule was defined according to previous results from phase I and II trials of single-agent Navitoclax in patients with solid tumors [31,32]. It was in line with the Navitoclax investigator's brochure. Patients initiated Navitoclax single-agent treatment at the daily dose of 150 mg orally given for a lead-in period lasting 7 to 14 days: in the case of platelet count was less than 50,000 /mm^3 from in the first 7 days, the treatment was stopped until the platelet count recovered to grade 0–1, and the lead-in period could be extended to a maximum of 14 days. Patients could only proceed from the lead-in period to the defined daily dose level of 250 mg for Cycle 1 Day 1 (that corresponded to Day 8 et 15 after first administration) and beyond if platelet count remained higher than 50,000 /mm^3 and platelets were stable or rising thereafter, in the absence of thrombocytopenia grade 3 or 4. For patients who developed thrombocytopenia grade 3 or higher (< 50,000/mm^3) during the lead-in period that recovered to grade 1 or less (≥ 75,000/mm^3) within a maximum of 14 days for the lead-in period, the daily dose was maintained at 150 mg for cycle 1 and further and was not increased. Cycle duration was defined as 21 days. Navitoclax treatment was administered until either cancer progression based on RECIST v1.1, unacceptable toxicity or the patient opted to withdraw from the study.

2.5. Dose adjustment and treatment discontinuation

All adverse events occurring during the active portion of therapy, or up to 30 days after the last dose of treatment, were graded by a numerical score according to the NCI's Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

Patients requiring a lead-in period longer than 14 days or a delay of more than 3 weeks during treatment cycles had to prematurely discontinue Navitoclax treatment. The targeted dose of Navitoclax was 250 mg daily; only one dose reduction was possible to the daily dose level of 150 mg. Patients requiring more than one dose reduction had to definitively discontinue the protocol therapy.

Navitoclax treatment had to be discontinued if the following Navitoclax-related toxicities occurred: thrombocytopenia, neutropenia, or non-hematologic toxicities grade 3 or higher. Administration of Navitoclax was also suspended for any clinically significant bleeding, defined as hemorrhage grade 2 or higher related to Navitoclax, whatever the platelet count. In the event of discontinuation for first occurrence of severe toxicities related to the experimental treatment, Navitoclax was re-introduced at the daily dose of 150 mg only in patients whose adverse events recovered to grade 1 or less.

2.6. Evaluations during study

Physical examinations and toxicity assessments were performed one and two weeks after Navitoclax initiation, and thereafter on day 1 of each cycle. Laboratory exams with complete blood cell count, chemistry and CA125 were also performed before each cycle. A cardiac evaluation with ECG was performed every 2 cycles as a systematic safety procedure. Tumor evaluation with CT scan was realized every 2 cycles.

2.7. Expression of tumor biomarkers

A centralized review of biomarker expression for relapsed disease before Navitoclax initiation and primary tumor sample (when available) was performed. Immunohistochemistry was used to assess expression of pro-apoptotic protein Bim, anti-apoptotic Bcl-2 family members (Bcl-x_L, Mcl-1) and phospho-ERK1/2. Immunohistochemistry was performed on paraffin-embedded tumor tissues using a Ventana Discovery XT autostainer on 4 μm-thick sections. Slides were deparaffinized with EZPrep buffer at 75 °C for 8 min and epitopes were unmasked at 95 °C for 8 min and 100 °C for 4 min in EDTA buffer. Sections were incubated for 40 min at 37 °C with Bim antibody (C34C5, # 2933 Cell Signaling, 1/150), Mcl-1 (Y37, #ab32087 Abcam, 1/1000), P-ERK (P-p44/42 MAPK (Erk1/2) (Thr202/Thr204) D13.14.4E, #4370 Cell Signaling,1/4000), Bcl-$x_{L/S}$ (#556361 BD Pharmingen 1/400), Bcl-2 (#M0887 Dako, 1/25), Noxa (#3665–100 Biovision, 1–25), Puma (D30C10, #12450 Cell Signaling, 1/50), Bid (#ab201754, Abcam,1/1000), Bax (#2774 Cell Signaling, 1–200), Bak (#6947 Cell signaling, 1/1500). Secondary antibody (Omnimap Rabbit) was incubated for 16 min at 37 °C. After washes, staining was performed with 3,3′-diaminobenzidine (DAB) and sections were counterstained with Hematoxylin. Whole slide images were digitized at 20 × (0,5 μm/pixel) using the ScanScope CS scanner (Leica Biosystems, Nussloch, Germany).

After immunodetection of the proteins of interest on tumor tissue samples, protein expression levels have been evaluated by an experienced pathologist (C. Blanc-Fournier) and classified as low, medium or high, in the same way as in our previous preclinical study [24]. This classification has been performed independently for each biomarker, since the staining intensity can strongly vary from a protein to another. For instance, Bcl-x_L expression can be very intense as compared to Bax or Puma expression. A low Bcl-x_L expression could thus correspond to a medium expression of PUMA or to a high expression of Bax. Examples of immunostainings observed for each biomarker in each of the three classes are presented in the results section.

2.8. Statistical considerations

This phase II trial was based on a Case & Morgan two-stage design [33]. The primary endpoint was PFS, defined as the delay between initiation of Navitoclax treatment and disease progression assessed according to RECIST v1.1 criteria, or death from any cause.

According to previous studies conducted in platinum-refractory/resistant ovarian cancer patients with previous cytotoxic treatments who received either pegylated liposomal doxorubicin (PLD) [34] or other novel anti-cancer drugs [35,36], median PFS varied from 2.7 to 5.3 months. We thus posited a median PFS of 2.5 months or less (H0) as non-acceptable and expected a median PFS of 4.5 months (H1). Assuming an exponential distribution of survival, the following assumptions were considered: median PFS ≤ 2.5 months is equivalent with a 3-month PFS rate ≤ 0.435 (H0) and median PFS ≥ 4.5 months is equivalent with a 3-month PFS rate ≥ 0.630 (H1).

Using the Case & Morgan [33] EDA two-stage design with a 10% one-sided alpha risk and a power of 85%, 19 assessable patients were needed to be included in the first step. Unless the interim analysis concluded that the study had to be stopped for futility, 22 additional patients had to be enrolled for a total of 41 assessable patients. To palliate around 10% of non-assessable patients, we planned to enroll a total of 46 patients over 24 months: 22 patients in the first step and 24 in the second step. Inclusions were not planned to be suspended to conduct the interim analysis, except if there were major limiting toxicities, including severe thrombocytopenia.

Qualitative variables are described by frequencies and percentages, and quantitative variables by medians and extreme values. Each *p*-value is accompanied with the corresponding test (Chi-squared for description of qualitative variables, log-rank or Cox model for time-to-event endpoints). Alpha risk level of 5% is retained for each test. Statistical analyses were performed by using R software, version 4.0.2.

Table 1
Patient and medical characteristics at baseline ($N = 46$).

	N = 46 (%)
Median age (years)	63 (range 38–80)
Primary cancer	
Ovarian	44 (96)
Primary Peritoneal	1 (2)
Fallopian Tube	1 (2)
Initial FIGO	
I/II	1 (2)
III	33 (72)
IV	12 (26)
ECOG PS	
0	20 (43)
1	26 (57)
BRCA mutations	
BRCA 1/2	7 (16)
Negative	25 (54)
Unknown	14 (30)
Prior lines of chemotherapy	
2	4 (9)
3	15 (32)
≥4	27 (59)
Prior treatment by bevacizumab	36 (78)
Time to relapse after end of last platinum-based chemotherapy*	3.8 (range 0.2–6.4)
Lines of nonplatinum-based chemotherapy between last platinum-based chemotherapy and Navitoclax	
0	24 (52)
1	10 (22)
≥2	12 (27)
Delay between end of last platinum-based regimen and first administration of Navitoclax (months)	7.6 (range 0.9–49.2)
Duration of Navitoclax treatment (days)	50 (range 2–281)

* 4 patients progressed during platinum-based chemotherapy.

3. Results

3.1. Patient characteristics

From January to September 2016, 47 patients were included in 13 French institutions of the GINECO group and 46 patients were treated by Navitoclax (one patient did not initiate Navitoclax treatment due to health status deterioration after inclusion). The patient and medical characteristics are summarized in Table 1. Median age of the enrolled patients was 63 years. All patients had a performance status of 0 (43%) or 1 (57%). Tumor localization was ovarian for most patients. At diagnosis, the International Federation of Gynecology and Obstetrics (FIGO) disease stage was 3 and 4 in almost all patients. Regarding eligibility criteria, serous histology of disease was documented in all cases. Patients received a median of 4 prior lines, and 91% of patients had received at least three previous lines. In 24 patients, the last treatment prior to Navitoclax administration included platinum; respectively 10 and 12 patients received one and ≥ 2 nonplatinum-based chemotherapies in the interval between platinum-based treatment and Navitoclax treatment. Concerning the last treatment by a platinum-based chemotherapy, relapse occurred within 3.8 months in median after the end of treatment for 42 patients, while 4 patients progressed during the treatment. Finally, Navitoclax administration started in median 7.6 months after the last platinum-based regimen dose.

3.2. Treatment description

Two patients out of 46 who initiated Navitoclax treatment stopped the monotherapy at the end of the lead-in period because of disease progression. Navitoclax could be pursued at the daily dose of 250 mg from Day 1 Cycle 1 in 36 patients (78%). The others experienced thrombocytopenia grade 3 or higher during the lead-in period and thus continued the treatment at the dose of 150 mg. A median number of two cycles of Navitoclax [0−11] was administered. The major reason for end of treatment was progressive disease (38 patients; 83%). Five patients (11%) discontinued treatment for toxicities (2 patients with grade 3 thrombocytopenia, one patient with grade 4 thrombocytopenia, one patient with grade 3 edema, one patient with grade 3 asthenia and grade 2 anorexia) and 3 patients (6%) for another reason.

3.3. Safety

Thrombocytopenia was the major expected side-effect, experienced by all patients except one. Severe thrombocytopenia occurred in 12 (26%) patients: grade 3 ($n = 11$) and grade 4 (n = 1). Grade 3/4 thrombocytopenia during the lead-in period resulted to pursue the Navitoclax at 150 mg in 8 patients. Thrombocytopenia grade 3 was responsible for treatment discontinuation in 3 patients (after 1, 2, and 3 completed cycles respectively). However, thrombocytopenia was not associated with clinically significant bleeding events and was reversible after

Table 2
Maximum grade of major toxicities observed during study (N = 46).

	All grades	Grade 1/2	Grade 3/4
	n (%)	n (%)	n (%)
Thrombocytopenia	45 *(98)*	33 *(72)*	12 *(26)*
Lymphopenia	37 *(80)*	28 *(61)*	9 *(20)*
Neutropenia	14 *(30)*	10 *(22)*	4 *(9)*
Leukopenia	22 *(48)*	19 *(41)*	3 *(7)*
Anemia	36 *(78)*	34 *(74)*	2 *(4)*
Fatigue	36 *(78)*	29 *(63)*	7 *(15)*
Alkaline Phosphatase increase	25 *(54)*	21 *(46)*	4 *(9)*
ASAT increase	23 *(50)*	20 *(44)*	3 *(7)*
GGT increase	21 *(46)*	16 *(35)*	5 *(11)*
High blood pressure	12 *(26)*	9 *(20)*	3 *(7)*

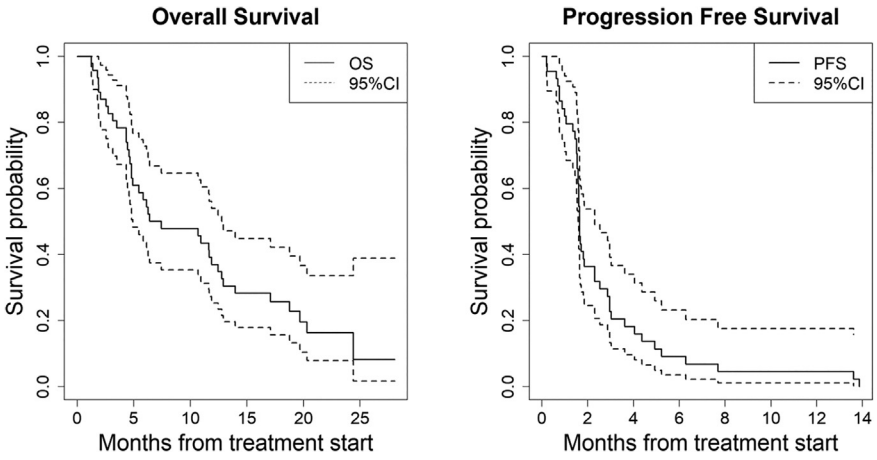

Fig. 1. Overall survival (left) and progression-free survival (right).

interruption of Navitoclax. No toxic death or other major toxicity was observed (a description of major adverse events is given in Table 2).

3.4. Efficacy analysis

Interim analysis was not performed since the total number of evaluable patients was reached faster than planned (9 months instead of 24 months) during data monitoring (inclusions were not suspended during this time, as planned in the protocol). Efficacy was thus evaluated at final analysis only. Among 47 patients included, 46 patients were assessable for efficacy. Median PFS was 1.64 months (95% confidence interval 95%CI: 1.58–2.30) (Fig. 1). PFS rate at 3 months was 22.7% [13.2–39.2]. The Z2 Case & Morgan test statistic was −4.86, i.e. much smaller than the minimal efficacy cut-off of 1.188. Median overall survival was 6.9 months [4.9–12.8]; 8 patients survived more than 2 years, from 24.4 to 48.4 months (Fig. 1). One partial response and 15 stable diseases were observed (Fig. 2).

No difference in PFS was observed according to the free interval between the last platinum-based chemotherapy (0–6 months vs. > 6 months, log-rank $p = 0.20$). Indeed, in patients with a free interval < 6 months ($n = 19$) and ≥ 6 months ($n = 25$), median PFS was 1.61 months [1.51–2.96] and 1.64 months [1.61–3.61], and 3-month PFS rate was 15.8 [5.6–44.6] and 28 [14.9–52.5], respectively. Likewise, PFS was not associated with proceeding or not to the daily dose level of 250 mg after the lead-in period (log-rank $p = 0.16$).

After Navitoclax, 12 patients received platinum (carboplatin in 8 patients, as single agent for 4 patients, or combined with gemcitabine (n = 2) or pegylated liposomal doxorubicin (n = 2); oxaliplatin in 4 patients, combined with gemcitabine for 3 of them) with 3 partial responses and 4 stable diseases (58%). The median response duration was 7 months [2–12] and the median delay from previous platinum-CT was 18 months.

3.5. Biomarker expression and clinical outcomes

Immunohistochemistry data were available in 36 patients. Protein expression was quantified as low, medium or high (Fig. 3).

Bim was highly expressed in 9 patients, 4 of them with clinical benefit (chi-squared $p = 0.68$, Table 3): 1 partial response and 3 stable disease. Among these 9 women, 7 had a low expression of Mcl-1 and/or phospho-ERK, of whom 4 had a partial response or stable disease, showing no evidence of a relation with clinical response (Fig. 4). BIM expression, alone or combined with Mcl-1 and/or p-ERK, was not found to be predictive of Navitoclax benefit. Similar results were obtained with other pro-apoptotic proteins, and no predictive signature (biomarkers expression panel) was identified. Concerning the targets of Navitoclax, 31 out of 34 tumors lacked Bcl-2 expression. Moreover, high expression of Bcl-x_L was observed in 10 out of 33 patients.

Concerning pro-apoptotic molecules (multidomains and BH3-only proteins), the expression of the apoptosis effector Bax was low in 32/

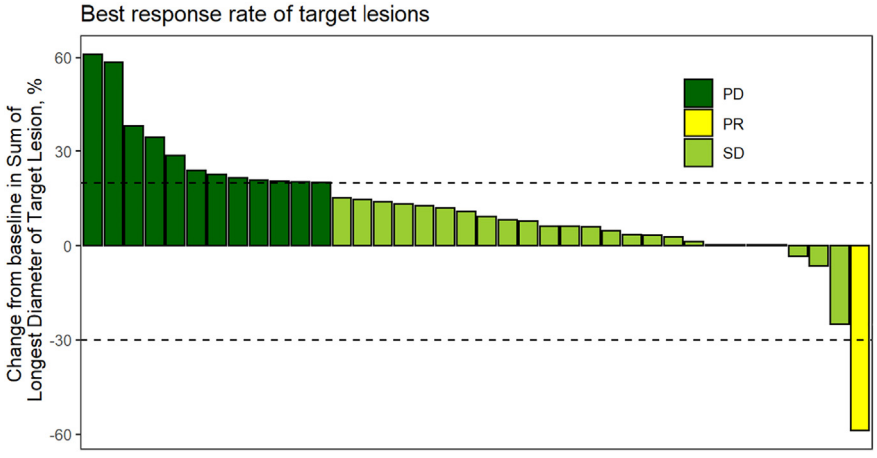

Fig. 2. Response of target lesions, defined by change from baseline in sum of diameters of target lesions. PD: Progressive Disease (≥ 20%), PR: Partial response (<−30%), SD: Stable Disease (from −30% to 20%).

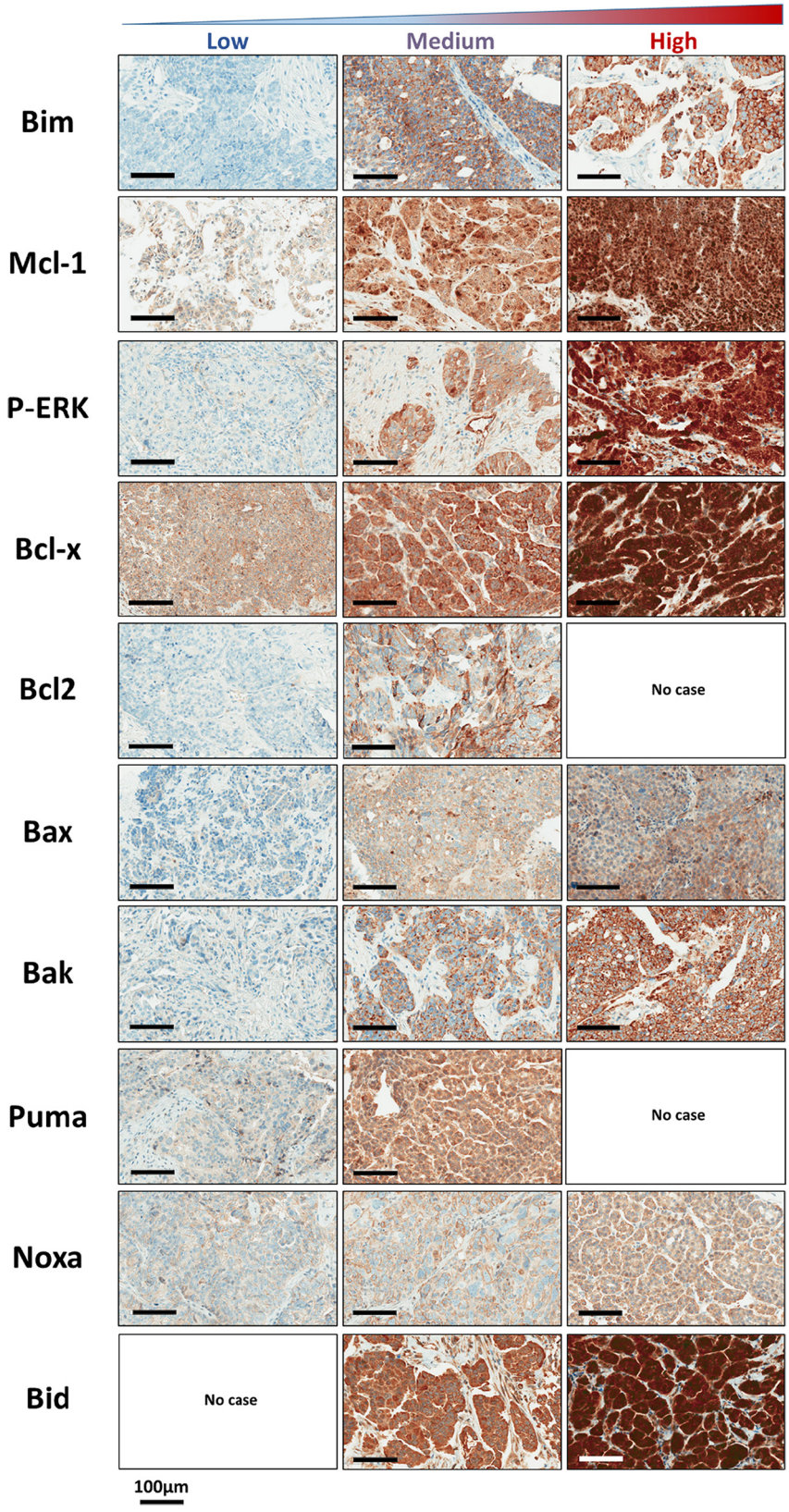

Fig. 3. Bcl-2 family proteins and P-ERK immunodetection performed on biopsies. Immunostaining classified as low, medium of high for each protein.

36 cases. Bak expression was low in 22/36 cases, without any correlation with response to treatment, either alone or combined with other data.

Otherwise, the BH3-only protein Puma was under-expressed whereas Bid was over-expressed in the samples, precluding the use of these proteins as predictive factors of the response to Navitoclax. BH3-

Table 3
Mcl-1: 5 missing data, Bcl-xL: 3 missing data, BID: 6 missing data, Response: 1 not available in 36 patients with IHC. *p-values obtained by Cox model.

Protein	Expression	Total	RESPONSE			PFS		OS	
			PD	PR/SD	Chi^2				
		N(%)	N(%)	N(%)	p	HR	p*	HR	p*
Bim	Low/Medium	27 (75)	18 (78)	8 (67)	0.68	1		1	
	High	9 (25)	5 (22)	4 (33)		0.74 [0.34–1.60]	0.45	0.94 [0.44–2.03]	0.88
Mcl-1	Low	13 (42)	10 (50)	3 (30)	0.44	1		1	
	Medium/High	18 (58)	10 (50)	7 (70)		0.75 [0.35–1.59]	0.45	1.11 [0.52–2.35]	0.79
p-ERK	Low/Medium	28 (78)	17 (74)	10 (83)	0.69	1		1	
	High	8 (22)	6 (26)	2 (17)		1.27 [0.57–2.83]	0.57	1.32 [0.58–2.99]	0.51
Bcl-xL	Low/Medium	23 (70)	14 (67)	9 (82)	0.44	1		1	
	High	10 (30)	7 (33)	2 (18)		0.99 [0.44–2.23]	0.98	0.89 [0.41–1.92]	0.77
BLC2	Low	35 (97)	23 (100)	11 (92)	0.34	1		1	
	Medium/High	1 (3)	0 (0)	1 (8)		0.46 [0.06–3.42]	0.45	1.27 [0.17–9.54]	0.81
Noxa	Low	27 (75)	17 (74)	9 (75)	1	1		1	
	Medium/High	9 (25)	6 (26)	3 (25)		1.24 [0.56–2.76]	0.59	1.09 [0.51–2.35]	0.82
Puma	Low	30 (83)	17 (74)	12 (100)	0.074	1		1	
	Medium/High	6 (17)	6 (26)	0 (0)		1.79 [0.70–4.61]	0.23	0.81 [0.33–1.97]	0.64
BID	Medium	7 (23)	3 (18)	4 (33)	0.4	1		1	
	High	23 (77)	14 (82)	8 (67)		1.28 [0.54–3.07]	0.57	0.95 [0.40–2.25]	0.91
Bax	Low	32 (89)	20 (87)	11 (92)	1	1		1	
	Medium/High	4 (11)	3 (13)	1 (8)		1.35 [0.47–3.91]	0.58	0.95 [0.33–2.74]	0.93
Bak	Low	22 (61)	15 (65)	7 (58)	0.97	1		1	
	Medium/High	14 (39)	8 (35)	5 (42)		0.56 [0.27–1.17]	0.12	0.43 [0.20–0.91]	0.028

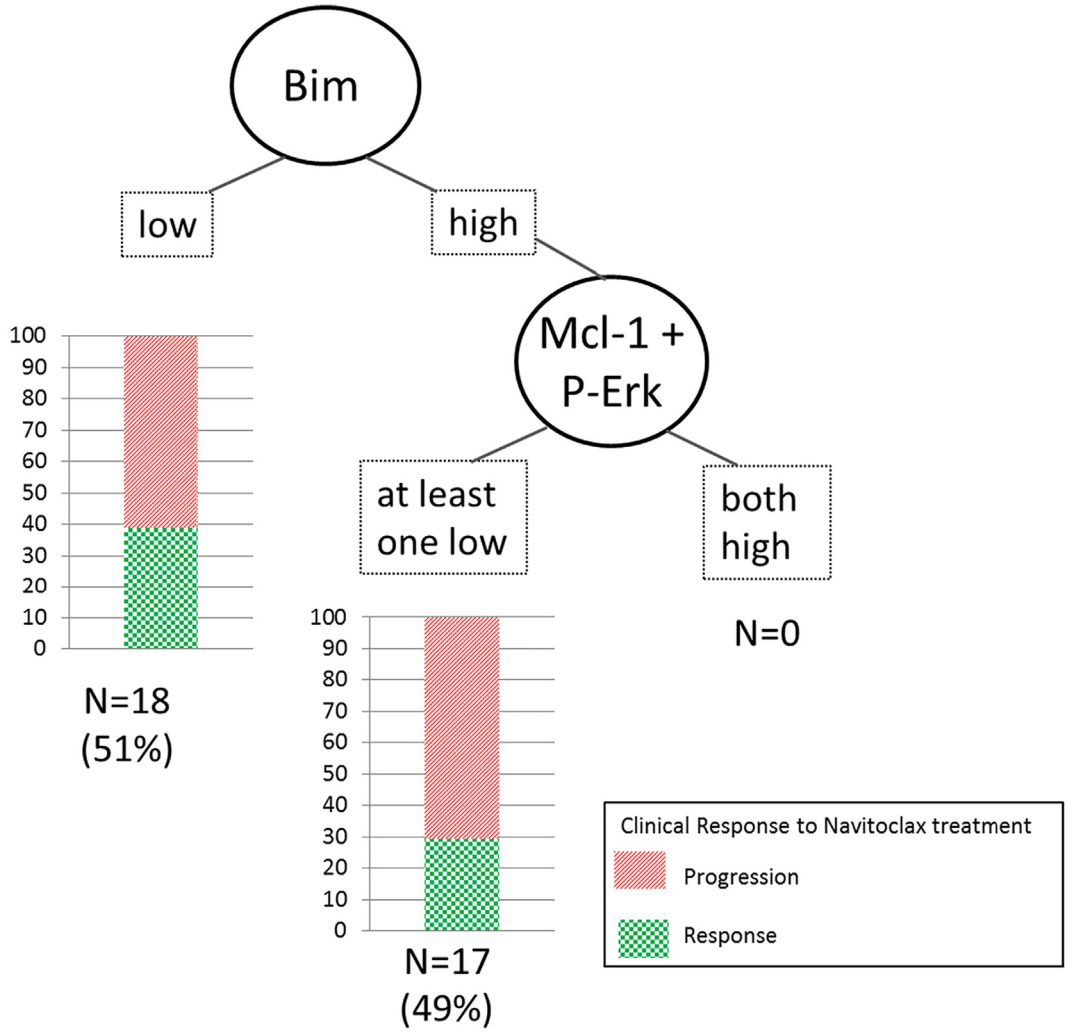

Fig. 4. Tumoral Bim, Mcl-1 and P-Erk expression and clinical response to Navitoclax treatment ($N = 35$).

only Noxa (able to be associated with Mcl-1 and to inhibit its anti-apoptotic activity) was low in 27/36 cases and did not correlate with clinical response.

4. Discussion

In this phase II trial, Navitoclax (ABT-263) monotherapy demonstrated a poor activity with an acceptable tolerance profile in heavily pretreated ovarian cancer patients. The study was prompted by the promising preclinical experience of BH3-mimetic agents administered ex vivo in ovarian tumor samples [24]. These preclinical results suggested the potential interest of Navitoclax used as a single agent, particularly in tumors with high expression of Bim and low expression of Mcl-1 and/or P-ERK. Navitoclax was therefore considered of potential interest in ovarian cancer.

Despite these encouraging preliminary preclinical data, we could not confirm the high clinical activity of this high-affinity BH3-mimetic agent. Apart from the setting of hematology, Navitoclax has mainly been trialed in small-cell lung cancer and our study was the first conducted in ovarian cancer patients. Our findings are consistent with previous findings from other clinical studies, suggesting that Navitoclax as monotherapy has limited efficacy in patients with solid tumors [28,31].

Interestingly, among the 12 patients who received platinum after Navitoclax progression, we observed 3 partial responses and 4 stable diseases. Of note, 4 of these 7 patients who benefitted from platinum had a period without platinum of more than 18 months, which may also explain the restoration of platinum sensitivity.

In the present study, the predictive signature previously established ex vivo (elevated Bim expression associated to a low Mcl-1 and/or P-ERK expression) was not confirmed on the tumor samples from the participating patients. Various factors may explain this discordance. First, 74% of the tumors in the present study exhibited low Bim expression versus 20% in our previous study performed on chemotherapy-naïve tumors. Second, 30% of the tumors expressed a high level of Bcl-x_L versus 56% in our previous study. In the present study, Bim expression, which is required for Navitoclax to be active, was found to be low, as well as that of the two other targets of Navitoclax. Indeed, Bcl-2 was not found to be expressed in this panel of patients, and Bcl-xL expression was mainly low, whereas it is usually described as being expressed at a high level in HGSOC in other studies [24,37], including our previous ones. The response of patients heavily pretreated by several lines of chemotherapy thus appears different from that of patients with chemo-naive tumors. However, we did not observe any substantial differences between the expression level of these proteins in archival samples and in biopsies taken at inclusion in the MONAVI trial (after lines of chemotherapy, data not shown). This suggests that these patients could present some intrinsic characteristics even prior first line chemotherapy that make them ineligible for a strategy based on the use of Navitoclax.

The levels of protein expression that we found could also be related to our technical conditions: e.g. the time between surgery and tissue fixation may affect ERK phosphorylation status and/or Mcl-1 expression. Such observations have been previously reported for several epitopes including P-ERK1/2 [38,39], where an extreme loss of immunoreactive P-ERK1/2 occurred during routine fixation of primary breast cancers. Levels of expression may thus differ between samples submitted to conventional paraffin embedding in institutional pathology laboratories, as in the MONAVI study, or to immediate fixation after surgery performed in a research unit, as in our previous ex vivo study. This strongly suggests the need to rapidly fix samples for biopsy used in IHC experiments. However, routine constraints could drastically limit the possible use of such approaches to predict the clinical response to Navitoclax. Other Bcl-2 family proteins might also impact the response to treatment and could participate in the predictive signature. However, neither Puma, Noxa, Bid, Bax or Bak expression were associated with the response to Navitoclax.

The poor efficacy of Navitoclax could partly be explained by the fact that all participating ovarian cancer patients were heavily pretreated. However, its limited efficacy may also result from its use as monotherapy. The clinical experience from trials conducted in the last few decades suggests that the combination of Bcl-2 inhibitors will be more effective than their use as a single agent.

For these reasons, various clinical trials have assessed Navitoclax in combination with chemotherapy drugs, based on the putative enhancement of the activity of chemotherapeutic agents by lowering their apoptotic threshold and potentializing the effects of cytotoxic agents in solid tumors [29,30,40]. Most were phase I trials and the major limiting toxicity was thrombocytopenia. This toxicity and the modest efficacy of several combinations of chemotherapy limited further investigations of Navitoclax [29,30,41]. Available data from the clinical trials suggest that the combination of Bcl-2 inhibitors with other drugs may be more effective than their use as a single agent, as previously suggested by preclinical studies performed in combination with platinum compounds [21] or various targeted therapies, including ours [24,42–45]. However, a phase I study recently performed in advanced solid tumors with Navitoclax combined with Docetaxel only reported a weak antitumor activity of this combination [46].

A recent review on drugs and clinical approaches targeting the antiapoptotic protein also highlights the low efficacy of Navitoclax and presents an overview of new Bcl-2 protein inhibitors currently under development [41]. In ovarian cancers, Bcl-2 seems to be rarely expressed, as is the case in the patients included in the present study and in those in our previous ex vivo study, so it might not be the most promising target. In contrast, Mcl-1, which is frequently overexpressed, could be another interesting target in ovarian carcinoma, especially since innovative drugs are now in development. Even if data are still poor to allow the use of these molecules in routine clinical practice, these studies offer new hope for the development of a BH3-mimetics-based strategy.

In summary, Navitoclax as a single-agent treatment has poor activity with an acceptable tolerance profile in heavily pretreated ovarian cancer patients. We failed to identify any predictive signature of the response to Navitoclax. However, our data also highlight the need to strengthen pre-analytical processes as well as quantification methodology associated to the analyzis of the expression of possible biomarkers, to avoid biases that could impede the identification of strong predictive signatures.

Previous presentation during congress

The results were presented in part during the European Society for Medical Oncology (ESMO) meetings in September 2017 and October 2018.

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Authors' contributions

Conception and design: F. Joly, L. Poulain, M. Fabbro, A. Leary.

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Declaration of Competing Interest

F. Joly: Abbvie for Navitoclax provision, consulting for Roche, GSK, Astra Zeneca, Clovis, MSD, Ipsen, Janssen, Astellas, Pfizer, Sanofi, BMS, Bayer.

M. Fabbro: Consulting for GSK, Astra-Zeneca, and Clovis Oncology.

P. Follana: GSK, Astra Zeneca, Clovis, MSD, Novartis, DAIICHI.

J.S. Frenel: Consulting for Novartis, Pfizer, Astra Zeneca, Lilly, Roche, BIOCAD, DAIICHI, GSK-Tesaro, Pierre Fabre, and Clovis oncology.

B.You: Consulting for MSD, Astra-Zeneca, GSK-TESARO, BAYER, Roche-Genentech, ECS Progastrine, Novartis, LEK, Amgen, Clovis Oncology, Merck Serono, BMS, SEAGEN, and Myriad.

J. Lequesne, J. Medioni, A. Lesoin, S. Abadie-Lacourtoisie, A. Floquet, L. Gladieff, C. Gavoille, E. Kalbacher, M. Briand, P.E. Brachet, F. Giffard, L-B. Weiswald, P.A. Just, C. Blanc-Fournier, A. Leconte, B. Clarisse, A. Leary, L. Poulain: no conflicts of interest to disclose in relation with this work.

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