





SARC025 Arms 1 and 2: A Phase 1 Study of the Poly (ADP-Ribose) Polymerase Inhibitor Niraparib With Temozolomide or Irinotecan in Patients With Advanced Ewing Sarcoma

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BACKGROUND: In preclinical Ewing sarcoma (ES) models, poly(adenosine diphosphate ribose) polymerase (PARP) inhibitors were identified as a potential therapeutic strategy with synergy in combination with cytotoxic agents. This study evaluated the safety and dosing of the PARP1/2 inhibitor niraparib (NIR) with temozolomide (TMZ; arm 1) or irinotecan (IRN; arm 2) in patients with pretreated ES. **METHODS:** Eligible patients in arm 1 received continuous NIR daily and escalating TMZ (days 2-6 [D2-6]) in cohort A. Subsequent patients received intermittent NIR dosing (cohort B), with TMZ re-escalation in cohort C. In arm 2, patients were assigned to NIR (days 1-7 [D1-7]) and escalating doses of IRN (D2-6). **RESULTS:** From July 2014 to May 2018, 29 eligible patients (23 males and 6 females) were enrolled in arms 1 and 2, which had 7 dose levels combined. Five patients experienced at least 1 dose-limiting toxicity (DLT) in arm 1 (grade 4 [G4] neutropenia for >7 days or G4 thrombocytopenia), and 3 patients experienced at least 1 DLT in arm 2 (grade 3 [G3] colitis, G3 anorexia, or G3 alanine aminotransferase elevation). The maximum tolerated dose was NIR at 200 mg every day on D1-7 plus TMZ at 30 mg/m² every day on D2-6 (arm 1) or NIR at 100 mg every day on D1-7 plus IRN at 20 mg/m² every day on D2-6 (arm 2). One confirmed partial response was observed in arm 2; the median progression-free survival was 9.0 weeks (95% CI, 7.0-10.1 weeks) and 16.3 weeks (95% CI, 5.1-69.7 weeks) in arms 1 and 2, respectively. The median decrease in tumor poly(ADP-ribose) activity was 89% (range, 83%-98%). **CONCLUSIONS:** The combination of NIR and TMZ or IRN was tolerable, but at lower doses in comparison with conventional cytotoxic combinations. A triple-combination study of NIR, IRN, and TMZ has commenced. *Cancer* 2021;127:1301-1310. © 2020 The Authors. *Cancer* published by Wiley Periodicals LLC on behalf of American Cancer Society This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

KEYWORDS: Ewing sarcoma, irinotecan, niraparib, poly(adenosine diphosphate ribose) polymerase (PARP) inhibition, temozolomide.

INTRODUCTION

Ewing sarcoma (ES) is an aggressive small round blue cell tumor; it is the second most common primary malignant tumor of bone in young adults and accounts for 40% of malignant bone tumors in children and adolescents. In 85% of cases, it is associated with the generation of the EWS-FLI1 fusion gene by a translocation of t(11;22)(q24;q12).¹ Despite the identification of a clear oncogenic driver and successful trials honing frontline, intensive multi-agent chemotherapy regimens, adequate therapy for advanced and refractory disease remains elusive with survival rates of 20% to 30% for metastatic disease and 60% to 70% for localized disease.²⁻⁴

Poly(ADP-ribose) polymerase 1 (PARP1) inhibitors have potential therapeutic value in ES according to preclinical studies. Almost simultaneously, PARP was demonstrated to be a potential target in ES via a screen for identifying genetic determinants of drug activity and to be involved mechanistically in the oncogene-dependent DNA damage

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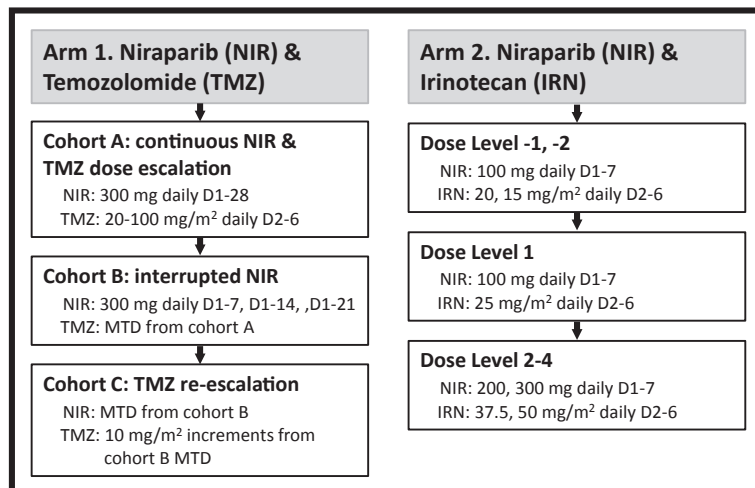


Figure 1. SARC025 study design. D indicates days; IRN, irinotecan; MTD, maximum tolerated dose; NIR, niraparib; TMZ, temozolomide.

response.^{5,6} Investigators also demonstrated that inhibition of PARP potentiates the activity of cytotoxic agents, particularly those that induce DNA damage through base excision repair.^{7,8} These agents include temozolomide and topoisomerase 1 inhibitors such as irinotecan; both agents are commonly used in ES. In addition, PARP inhibitors are able to trap PARP1 and PARP2 enzymes at damaged DNA.^{9,10} These trapped PARP-DNA complexes are more cytotoxic than unrepaired single-strand breaks caused by PARP inactivation, and this argues that PARP inhibitors act in part as poisons that trap the PARP enzyme on DNA.

We designed SARC025 (NCT02044120) to evaluate the safety and toxicity of niraparib, a potent and selective PARP inhibitor with potent PARP-trapping properties, in combination with temozolomide (arm 1) and subsequently irinotecan (arm 2) in patients with advanced ES.

MATERIALS AND METHODS

Study Design

This was a multicenter, phase 1 study of 2 sequential arms combining niraparib with temozolomide (arm 1) and niraparib with irinotecan (arm 2; Fig. 1). The primary objectives were to determine dose-limiting toxicities (DLTs) and the maximum tolerated dose (MTD) of niraparib combined with escalating doses of temozolomide or irinotecan. The secondary objectives included an evaluation of the objective response rate (ORR) by the Response Evaluation Criteria in Solid Tumors

(RECIST; version 1.1), progression-free survival (PFS), duration of response, and pharmacodynamic markers of responses to PARP inhibition. The study protocol was approved by appropriate institutional review boards at US centres and ethical committee approval in the UK (16/LO/0493).

Design for arm 1

Because the ideal dosing and schedule of niraparib and temozolomide in this population were not yet understood, the study was designed to evolve rapidly as data were collected. Initially, the trial began with cohort A and evaluated continuous niraparib (days 1-28 of a 28-day cycle) at 300 mg daily on the basis of the recommended adult phase 2 dose.¹¹ Temozolomide was initiated at 20 mg/m² (days 2-6 [D2-6]) to replicate the schedule in ES protocols, with niraparib administered 1 day earlier to inhibit PARP activity prior to the cytotoxic agent and for a pharmacodynamic evaluation of the inhibitor alone. If the MTD occurred within the first 3 dose levels, intermittent niraparib dosing would be explored in cohort B with a 3+3 model. After the MTD of cohort B was identified, cohort C was to evaluate the dose and schedule of niraparib in cohort B with escalating doses of temozolomide (Fig. 1).

Design for arm 2

On account of hematologic toxicities observed in arm 1, arm 2 was sequentially opened to investigate niraparib in combination with irinotecan because it is associated with less myelosuppression than temozolomide. Irinotecan was commenced at 25 mg/m² (days 2-6). A 3+3 design was

used for dose escalation with a dose expansion cohort of 9 patients to evaluate further for toxicity and preliminary evidence of efficacy (Fig. 1).

Patient Eligibility

Eligible patients had histologically confirmed advanced ES with EWS translocation by fluorescence in situ hybridization or reverse transcriptase–polymerase chain reaction. Requirements included an age ≥ 13 years, no known curative treatment, an Eastern Cooperative Oncology Group performance status of 0 to 2, 1 or more prior chemotherapy regimens, measurable disease according to RECIST (version 1.1), a life expectancy ≥ 3 months, and adequate hematologic (absolute neutrophil count $\geq 1.0 \times 10^9/L$, hemoglobin > 9 g/dL, and platelets $> 150 \times 10^9/L$) and renal function (serum creatinine ≤ 1.5 times the institutional upper limit of normal).

Patients who were previously treated with a PARP inhibitor or had a QTC_F value > 480 milliseconds, a known history of myelodysplasia, or active central nervous system disease were excluded.

Safety Assessments

Safety was monitored with weekly physical examinations and laboratory tests (complete blood counts and a comprehensive metabolic panel were performed at least twice weekly for the first 2 cycles). Electrocardiography, urinalysis, and pregnancy testing were performed before and at the end of treatment and also as clinically indicated. Adverse events were monitored continuously and were graded with the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 4).

Evaluation of Tumor Response

Tumor assessments were performed by investigators using RECIST (version 1.1). Baseline radiology imaging studies were required within 28 days of cycle 1 day 1. Thereafter, tumor assessments were performed every 2 cycles (8 weeks) for the first 6 cycles and then every 2 to 3 cycles.

Biomarker Assessments

PARP-specific pharmacodynamic studies

Peripheral blood (PB) and tumor samples were taken to compare poly(ADP-ribose) (PAR) levels as a pharmacodynamic biomarker of PARP1/2 catalytic activity. PB was taken before treatment, on cycle 1 day 2 (4 hours after the dose), on cycle 2 day 8, and at the end of treatment. Patients without medical contraindications

TABLE 1. Patient Demographics

Age at study entry, median (range), y	25 (13-50)
Sex, No. (%)	
Female	6 (21)
Ethnicity, No. (%)	
Hispanic or Latino	2 (7)
Race, No. (%)	
American Indian or Alaskan Native	1 (3)
Asian	3 (10)
Native Hawaiian or other Pacific Islander	1 (3)
White	23 (79)
Other	2 (7)
ECOG PS, No. (%)	
0	11 (38)
1	15 (52)
2	3 (10)
Primary tumor origin, No. (%)	
Osseous	23 (79)
Extra-osseous	6 (21)
Translocation confirmation, No. (%)	
RT-PCR	12 (41)
FISH	15 (52)
Not available	2 (7)
Age at initial diagnosis, median (range), y	20 (6-45)
Time to metastatic disease, median (range), y	1.2 (0-5.9)
Time from diagnosis to study entry, median (range), y	3.1 (0.2-12)
Metastatic disease at time of diagnosis, No. (%)	7 (26)
Tumor location at diagnosis, No. (%)	
Extremity	8 (28)
Pelvis	7 (24)
Thorax	4 (14)
Other	10 (34)
Prior treatments	
Prior systemic regimens, median (range)	4 (1-12)
Prior temozolomide (arm 1, n = 17)	10 (59)
Prior irinotecan (arm 2, n = 12)	7 (58)
Prior surgical resection, No. (%)	28 (97)
Prior surgeries, median (range)	2 (0-8)
Prior primary site tumor resection, No. (%)	17 (59)
Prior radiation, No. (%)	27 (93)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; FISH, fluorescence in situ hybridization; PS, performance status; RT-PCR, reverse transcriptase–polymerase chain reaction.

underwent tumor biopsies before treatment and on treatment on cycle 2 day 8. Tumor and PB PAR contents were quantified with a validated enzyme-linked immunosorbent assay as previously described.^{12,13}

Statistical Analysis

For the dose escalation in cohort A of arm 1 of the trial, a continual reassessment method was used to allow for continuous evaluation and enrollment of multiple dose levels within 1 cohort. This was followed by a planned 3+3 design for subsequent cohorts. DLTs were defined as any of the following events occurring during the first cycle of treatment and attributable to either drug: 1) any drug-related death, 2) any grade 3 or 4 treatment-related nonhematologic toxicity (excepting grade 3 electrolyte abnormalities that resolved to less than grade 1 or the baseline within 72 hours and grade 3 nausea, vomiting, and diarrhea if controlled within 72 hours), 3) grade 4

TABLE 2. Dose Levels and DLTs Observed

Dose Level	No. of Patients	Doses	% Dose of Prescribed NIR Received	No. of Cycles, Median (Range)	DLTs Observed
Arm 1: NIR plus TMZ					
A1	3	NIR at 300 mg qd D1-28 TMZ at 20 mg/m ² qd D2-6	62 (52-62)	2 (2-3)	G4 thrombocytopenia (n = 2) G4 neutropenia (n = 1)
B1	3	NIR at 300 mg qd D1-7 TMZ at 20 mg/m ² qd D2-6	63 (56-100)	3 (2-3)	G4 thrombocytopenia (n = 2) G4 neutropenia (n = 1)
B4	3	NIR at 200 mg qd D1-7 TMZ at 20 mg/m ² qd D2-6	100	2 (1-2)	None
B5	5	NIR at 200 mg qd D1-14 TMZ at 20 mg/m ² qd D2-6	75 (75-100)	2 (1-2)	G4 thrombocytopenia (n = 2) G4 neutropenia (n = 1)
C1	3	NIR at 200 mg qd D1-7 TMZ at 30 mg/m ² qd D2-6	100 (93-100)	2 (1-2)	None
Arm 2: NIR plus IRN					
-1	9	NIR at 100 mg qd D1-7 IRN at 20 mg/m ² qd D2-6	100	3 (1-13)	None
1	3	NIR at 100 mg qd D1-7 IRN at 25 mg/m ² qd D2-6	100 (67-100)	2 (1-17)	G3 colitis (n = 1) G3 anorexia (n = 1) G3 ALT elevation (n = 1)

Abbreviations: ALT, alanine aminotransferase; D, days; DLT, dose-limiting toxicity; G, grade; IRN, irinotecan; NIR, niraparib; qd, every day; TMZ, temozolomide.

neutropenia for >7 days, 4) grade 3 or 4 febrile neutropenia with an elevated temperature on 2 occasions or with a documented bacterial infection, 5) grade 4 thrombocytopenia, and 6) any adverse event leading to a dose interruption for >7 days. The MTD was determined as the dose level immediately below the lowest dose with ≥ 1 of 3 or ≥ 2 of 6 with a DLT.

Relevant results pertaining to toxicity, MTD, efficacy, and laboratory correlates were examined in an exploratory and hypothesis-generating fashion. Adverse events (overall and by dose level) and objective responses were tabulated and summarized. Time-to-event variables were summarized descriptively. The Kaplan-Meier estimator was used to generate survival estimates and curves. The levels of PAR and PARP activity were summarized descriptively and plotted along time by dose levels and as a whole.

RESULTS

Patient Characteristics

Between July 2014 and May 2018, 29 eligible patients were treated. Baseline characteristics are summarized in Table 1.

Dose Escalation

Arm 1

Initial dose escalation in cohort A at dose level 1 (A1) resulted in hematologic DLTs (Table 2) requiring early discontinuation of continuous niraparib dosing in cycle 1 on days 16, 7, and 15 for the first 3 patients. Subsequently, cohort B with intermittent niraparib dosing was amended to include lower niraparib dosing based

on toxicities observed in cohort A (Table 2). Niraparib at 200 mg (days 1-7 [D1-7]) and temozolomide at 20 mg/m² (D2-6) were tolerable and determined to be the MTD within cohort B. The study progressed to cohort C without DLTs observed in 3 patients with temozolomide at 30 mg/m² (D2-6). Because of a lack of clinical activity and under the hypothesis that further escalation would be constrained on account of toxicity, additional patients were subsequently enrolled in arm 2.

Arm 2

DLTs were observed in 3 patients at dose level 1: niraparib at 100 mg (D1-7) and irinotecan at 25 mg/m² (D2-6; Table 2). There were no DLTs observed at dose level -1: niraparib at 100 mg (D1-7) and irinotecan at 20 mg/m² (D2-6).

Safety and Tolerability

All 29 patients were evaluable for toxicities. Treatment-emergent adverse events across all cycles with a frequency of more than 10% are listed in Table 3. Greater hematologic toxicity was observed in arm 1, and greater gastrointestinal toxicity was observed in arm 2.

Correlative Studies

Nineteen patients (66%) had archival tumor samples available for correlative analysis, 21 (72%) had baseline biopsies performed, and 15 (52%) had on-treatment biopsies. A PAR analysis of tumor samples taken before and after treatment (cycle 2 day 8) was performed in a subset of patients. This demonstrated a significant reduction in PAR activity in all samples tested (median, 89%; range, 83%-98%), with no difference observed between 200 and 100 mg of

TABLE 3. Arm 1 and Arm 2 Adverse Events, Regardless of Attribution, Occurring in $\geq 10\%$ in at Least 1 Arm

Adverse Event	Arm 1 (n = 17): NIR + TMZ		Arm 2 (n = 12): NIR + IRN	
	All, No. (%)	Grade 3 or 4, No. (%)	All, No. (%)	Grade 3 or 4, No. (%)
Hematologic				
Anemia	8 (47)	2 (12)	5 (42)	
Leukopenia	9 (54)	3 (18)	4 (33)	1 (8)
Lymphopenia	8 (47)	2 (12)	3 (25)	1 (8)
Neutropenia	6 (35)	3 (18)	5 (42)	2 (17)
Thrombocytopenia	13 (77)	6 (35)	5 (42)	1 (8)
Cardiovascular disorders				
Hypotension	3 (18)		1 (8)	
Sinus tachycardia	3 (18)		1 (8)	
Constitutional				
Anorexia	4 (24)		7 (58)	1 (8)
Chest pain	1 (6)		2 (17)	
Fatigue	8 (47)		6 (50)	
Fever	3 (18)		3 (25)	
General disorders and administration site conditions, other	1 (6)	1 (6)	2 (17)	
Musculoskeletal disorders, other	5 (29)	1 (6)	2 (17)	
Pain	3 (18)		1 (8)	
Pain in extremity	3 (18)	1 (6)	1 (8)	
Weight loss	4 (24)		1 (8)	
GI disorders				
Abdominal pain	4 (24)	2 (12)	7 (58)	
Colitis			4 (34)	2 (17)
Constipation	5 (29)		3 (25)	
Diarrhea	3 (18)		10 (84)	2 (17)
Dyspepsia	2 (12)			
GI disorders, other	1 (6)		2 (17)	
Mucositis			3 (25)	
Nausea	12 (71)	1 (6)	12 (100)	1 (8)
Vomiting	9 (54)	2 (12)	7 (58)	1 (8)
Hepatobiliary disorders				
Elevated ALT	6 (35)		2 (17)	1 (8)
Elevated Alk Phos	8 (47)			
Elevated AST	5 (29)		1 (8)	
Infections, other				
	2 (12)		2 (17)	
Metabolism and nutrition disorders				
Hyperglycemia	4 (24)		1 (8)	
Hypoalbuminemia	5 (29)			
Hypocalcemia	2 (12)		1 (8)	
Hyponatremia	3 (18)			
Hypophosphatemia	1 (6)		2 (17)	
Nervous system disorders				
Dizziness	1 (6)		2 (17)	
Dysgeusia	1 (6)		2 (17)	
Headache	3 (18)		2 (18)	
Psychiatric disorders				
Anxiety	2 (12)		1 (8)	
Insomnia	5 (30)		1 (8)	
Renal and urinary disorders				
Elevated creatinine	5 (29)		1 (8)	
Renal/urinary disorders, other	2 (12)			
Respiratory disorders				
Cough	3 (18)		2 (17)	
Dyspnea	2 (12)		1 (8)	
Respiratory disorders, other	2 (12)			
Skin and subcutaneous tissue disorders, other				
Rash, maculopapular	3 (18)		1 (8)	

Abbreviations: Alk Phos, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GI, gastrointestinal; IRN, irinotecan; NIR, niraparib; TMZ, temozolomide.

niraparib (Fig. 2A). The reduction in PAR activity was less consistent in PB at the same time point (median, 62%; range, 41%-72%) with a correlation coefficient of 0.77 (Fig. 2B). All analyzed PB samples demonstrated PARP

inhibition on c1D2 4 hours after the dose (Supporting Fig. 2). Recovery of PARP activity was observed before cycle 2 treatment, with further inhibition demonstrated to be sustained to day 8 of cycle 2 after D1-7 niraparib.

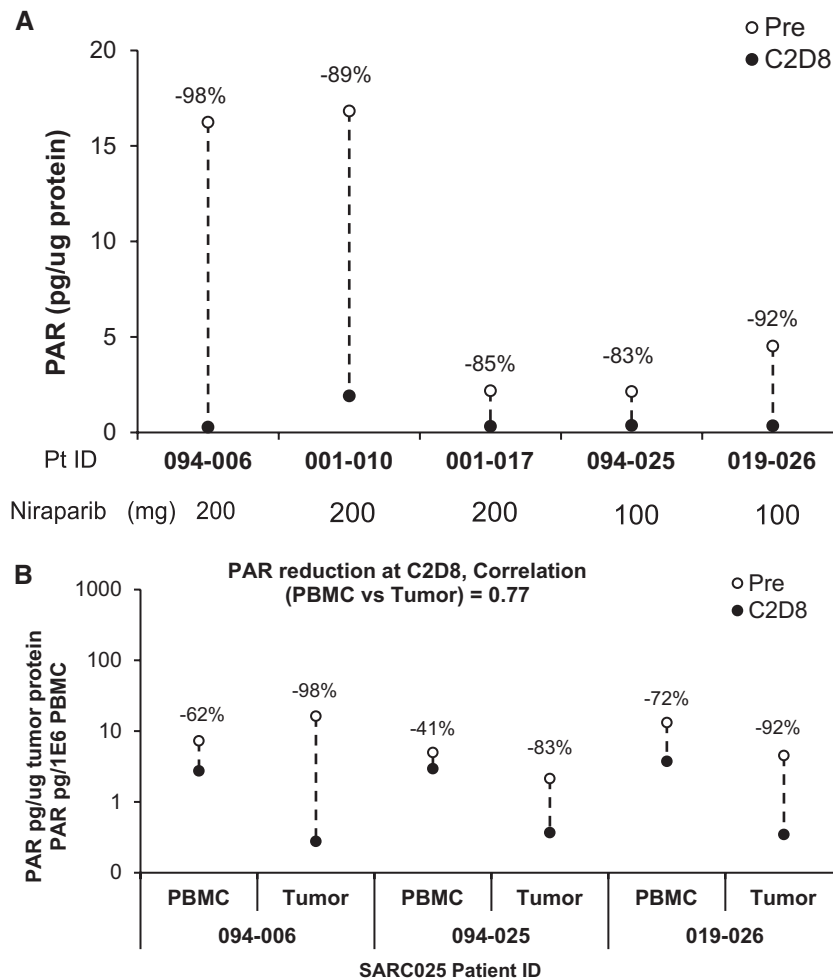


Figure 2. PAR levels analyzed in a subset of patients with tumors that were obtained before treatment and on C2D8. (A) There was a significant reduction in PAR in tumor samples after niraparib exposure across both 100- and 200-mg dose levels. (B) Decreases in PAR levels in peripheral blood were less consistent. The PAR reduction correlation on C2D8 (peripheral blood vs tumor) was 0.77. C2D8 indicates cycle 2 day 8; PAR, poly(adenosine diphosphate ribose); PBMC, peripheral blood mononuclear cell.

Outcomes

Patients in arm 1 received a median of 2 cycles (range, 1-3 cycles) with a median PFS of 9.0 weeks (95% CI, 7.0-10.1 weeks; Fig. 3A). In arm 2, 12 patients received a median of 2 cycles (range, 1-16 cycles). One patient achieved a partial response and 6 patients had stable disease for an ORR of 8.33%; the median PFS was 16.3 weeks (95% CI, 5.1-69.7 weeks; Fig. 3B). Two patients remained on study for more than 1 year (16 and 18 months, respectively),

DISCUSSION

The management of patients with recurrent, refractory ES is typically approached with sequential combination chemotherapy regimens with modest activity. The

rEECur study, an international, randomized trial comparing 4 commonly used regimens in the second-line setting (including a combination of irinotecan and temozolomide), preliminarily reported an ORR of 18%, median PFS of 4.7 months, and overall survival of 13.7 months across all treatments.¹⁴

PARP inhibition has proven to be a beneficial strategy in BRCA-mutated breast and ovarian cancer, where accumulating DNA damage in the setting of defective homologous recombination combined with PARP inhibitor-induced deficiency in base excision repair leads to synthetic lethality of the cancer cell.^{15,16} Similarly, SARC025 was designed to capitalize on the biology of ES and the specific mechanistic effects of PARP inhibitors on EWS-FLI1 transcription. Preclinically, a combination

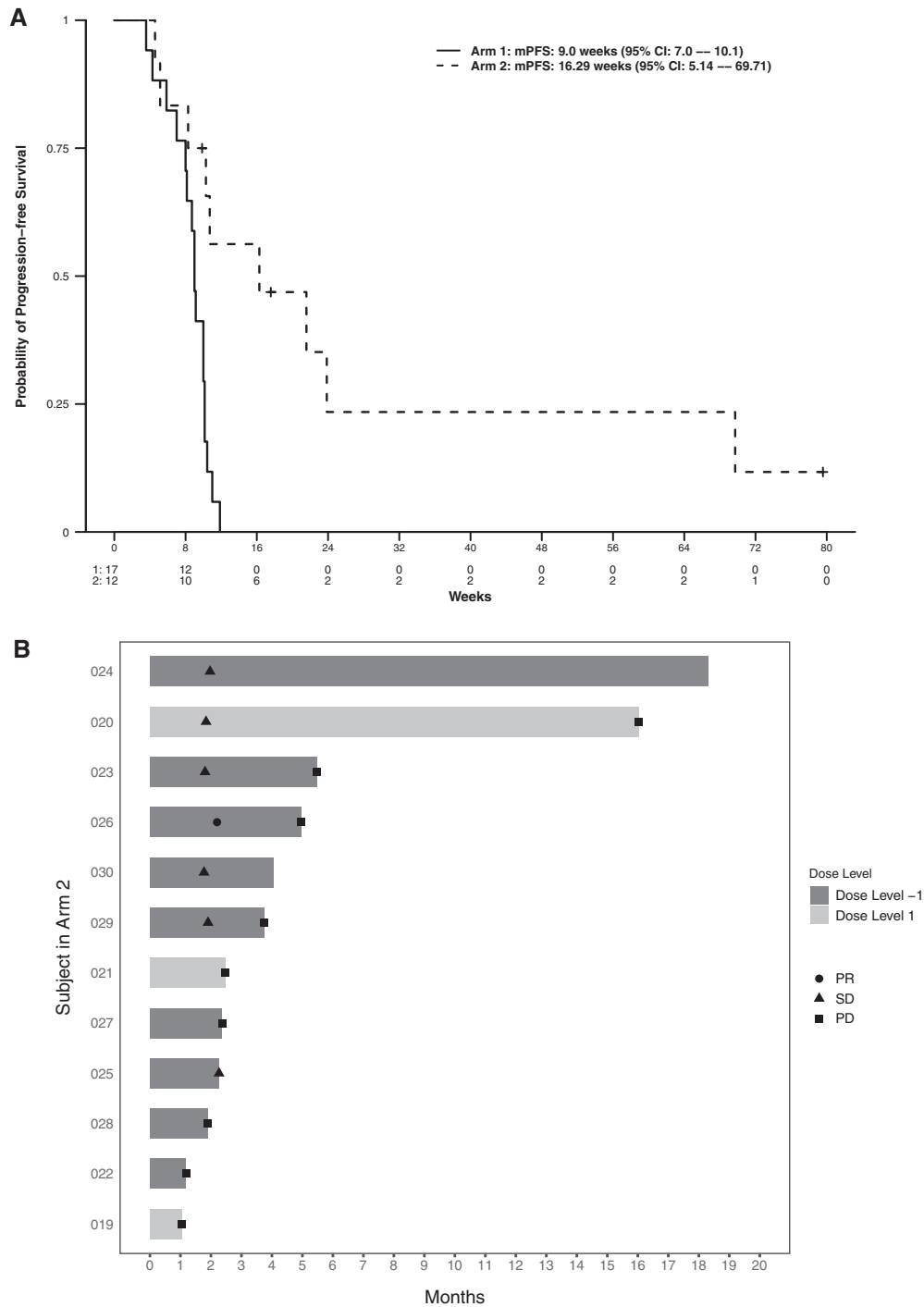


Figure 3. (A) Kaplan-Meier curves of progression-free survival for patients treated in arms 1 and 2. (B) Swimmer's plot of outcomes for patients treated in arm 2. mPFS indicates median progression-free survival; PD, progressive disease; PR, partial response; SD, stable disease.

with agents that induce DNA damage results in significant synergy, with both temozolomide and irinotecan being appropriate candidates.⁵ However, prior early-phase clinical trials evaluating PARP combination therapy with

cytotoxic agents were associated with significant toxicity; thus, the first challenge was to define the most appropriate schedule and dosing of both agents.¹⁷ Another challenge was conducting a tumor-specific phase 1 study of a rare

disease. This was strongly desired to best determine the toxicities in this typically heavily pretreated population. It was also a pertinent consideration on account of the age spectrum of the patient population, which precluded its falling within traditional pediatric or adult phase 1 clinical trial structures. Importantly, we were able to include patients aged 13 years or older.

Based on the known interaction with EWS-FLI1 and *in vivo* models supporting prolonged PARP inhibition resulting in greater efficacy, a strategy of continuous PARP inhibition was initially attempted with a higher dose of a PARP inhibitor and a low dose of temozolomide used.^{8,18} Within the first cohort of arm 1, this was found not to be a viable strategy. Dramatic and prolonged thrombocytopenia and neutropenia were observed in the first cycle, and patients were unable to remain on continuous niraparib (Supporting Fig. 1). Dose level modifications were made to allow for interrupted dosing of niraparib and subsequent escalation of temozolomide (arm 1). At the completion of arm 1, temozolomide was being dosed at 30% of the standard along with niraparib at 66% of its single-agent dosing for 7 days of a 28-day cycle.

In view of the hematologic toxicity observed in arm 1, arm 2 commenced with a lower starting dose of niraparib and with irinotecan administered at 25 mg/m² (50% of the standard when given in combination with temozolomide). Unfortunately, this too resulted in DLTs at the first dose level (here mainly gastrointestinal ones). Dose level -1 was well tolerated without DLTs across 9 patients, including those treated as part of dose expansion.

An additional objective of the study was to determine the feasibility of obtaining tumor biopsies in a patient population that spanned the spectrum of children and adults and their use for pharmacodynamic and biomarker analysis. Not all institutional review boards approved an on-treatment biopsy in patients under 18 years, so these were mandated only in adult patients. However, overall, the approach proved feasible and acceptable to patients and their families, with 21 of the 29 patients (72%) undergoing pretreatment biopsies, including 2 of the 3 patients under the age of 18 years. Fifteen of the 29 patients (52%) were also able to undergo on-treatment biopsies, with exceptions made for patients with medical contraindications or those coming off the study before the biopsy time point. The measurement of PAR activity was performed in a select subset of patients on account of limited resources, and it demonstrated excellent inhibition of PARP catalytic activity across both the 200- and 100-mg dose cohorts. This was comparable to data observed in other trials of PARP inhibitors and thus supported the

niraparib dosing strategy in arm 2, and it provided reassurance for ongoing study conduct.^{12,19} An additional correlative analysis including PARP expression, Schlafen 11, and R-loops, recently reported as being determinants of PARP and irinotecan sensitivity, is ongoing as part of a wider analysis and will be reported separately.^{20,21}

The PFS in arm 1 was 9.0 weeks across all cohorts, compared to 6.4 weeks with single-agent olaparib in a similar group of patients and approximately 7.8 weeks with inactive agents such as oral treosulfan.^{22,23} Efficacy was more promising in arm 2, with 6 patients (50%) achieving a partial response or stable disease with a median PFS of 16.3 weeks, and this included 2 patients who remained on study for more than 1 year. A benefit was observed in both irinotecan-naïve patients and those with previous exposure. No clinical features correlated with benefit in this small group of patients. The PFS was inferior to that demonstrated within the rEECur study; however, patients in our study had received a median of 4 prior therapies.

Despite the excitement generated by promising preclinical work, PARP inhibition in ES now garners less enthusiasm. The reason that greater activity has not been seen with single-agent or combination PARP inhibition is not completely understood. The greatest synergistic activity preclinically was observed with PARP inhibitors in combination with both irinotecan and temozolomide.⁸ It is possible that synergistic toxicities simply preclude achieving the effective prolonged dose exposure required for clinical activity, although extensive pretreatment in the ES population likely contributed to challenges with tolerance and/or therapy resistance. Heisey et al²⁴ recently reported that chemotherapy-resistant ES cell lines were also resistant to PARP inhibition. Expression of BCL-2 and BCL-X₁ was associated with resistance, with a BCL-2/X₁ inhibitor overcoming that resistance. Both PARP-DNA trapping and inhibition of PARP catalytic activity have been demonstrated to play roles in determining synergy with DNA damaging agents, such that differences among PARP inhibitors may contribute to efficacy and toxicity.¹⁰ A recent pediatric phase 1/2 study of talazoparib given in combination with temozolomide also demonstrated hematologic toxicity to be dose-limiting, with no responses in ES despite adequate PARP inhibition.²⁵

Noncytotoxic agents may potentially be better partners for PARP inhibition in ES. DNA repair in ES is dependent on DNA damage response pathways, particularly through ataxia telangiectasia and Rad3-related protein (ATR), which is activated in response to replication stress, a notable feature of ES through EWS-FLI1-mediated

increased transcription.²⁰ An ATR-PARP inhibitor combination by dual DNA repair pathway inhibition offers an attractive strategy.

This study reported the safety and tolerability of a potent PARP inhibitor with temozolomide or irinotecan, agents commonly used to treat patients with ES. Because hematologic and gastrointestinal toxicities were dose-limiting in arms 1 and 2, respectively, and appeared to be nonoverlapping, there is an additional arm investigating triple therapy. On the basis of preclinical data suggesting maximum synergy with the 3 agents, we hypothesize that with lower doses of all 3 agents, there may be less hematologic and gastrointestinal toxicity with the potential for synergistic efficacy. However, whether this will offer any advantage over higher dose, standard temozolomide and irinotecan therapy remains to be determined.

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CONFLICT OF INTEREST DISCLOSURES

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AUTHOR CONTRIBUTIONS

Rashmi Chugh: Conceptualization, data curation, formal analysis, writing—original draft, writing—review and editing, and approval of the final version of the manuscript. **Karla V. Ballman:** Conceptualization, data curation, formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Lee J. Helman:** Conceptualization, data curation, formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Shreyaskumar Patel:** Conceptualization, data curation, formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Jeremy S. Whelan:** Conceptualization, data curation, formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Brigitte Widemann:** Conceptualization, data curation, formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Yao Lu:** Formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Douglas S. Hawkins:** Conceptualization, data curation, formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Leo Mascarenhas:** Conceptualization, data curation, formal analysis, writing—review and editing, and approval of the final version of the manuscript. **John W. Glod:** Conceptualization, data curation, formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Jiuping Ji:** Formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Yiping Zhang:** Formal analysis, writing—review and editing, and approval of the final version of the manuscript. **Denise Reinke:** Conceptualization, funding acquisition, project administration, supervision, writing—review and editing, and approval of the final version of the manuscript. **Sandra J. Strauss:** Conceptualization, data curation, formal analysis, writing—original draft, writing—review and editing, and approval of the final version of the manuscript.

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