A Phase I/II Trial of Oral SRA737 (a Chk1 Inhibitor) Given in Combination with Low-Dose Gemcitabine in Patients with Advanced Cancer

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ABSTRACT

Purpose: This was a Phase I/II trial of the novel checkpoint kinase 1 (Chk1) inhibitor SRA737 given in combination with gemcitabine. Its objectives were to establish the safety profile, recommended Phase 2 dose (RP2D), pharmacokinetics profile, and clinical activity of SRA737.

Patients and Methods: Patients with advanced solid tumors were enrolled into dose-escalation cohorts and treated in 28-day cycles with oral SRA737 on days 2, 3, 9, 10, 16, and 17, and intravenous gemcitabine on days 1, 8, and 15. Treatment was continued until progression. Each expansion cohort included up to 20 patients with specific genetically defined tumors.

Results: The RP2D was determined to be 500 mg SRA737 combined with low-dose (250 mg/m^2) gemcitabine. Of 143 enrolled patients, 77 were treated at doses of at least 500 mg SRA737 combined with 250 mg/m^2 gemcitabine. Common toxicities of nausea, vomiting, fatigue, and diarrhea were primarily mild to moderate, and rarely led to treatment discontinuation. Anemia, neutropenia, and thrombocytopenia were grade ≥3 in 11.7%, 16.7%, and 10% of patients treated at the RP2D, respectively. The objective response rate (ORR) was 10.8% overall and notably the ORR in anogenital cancer was 25%. Partial tumor responses were observed in anogenital cancer, cervical cancer, high-grade serous ovarian cancer, rectal cancer, and small cell lung cancer.

Conclusions: SRA737 in combination with low-dose gemcitabine was well tolerated with lower myelotoxicity than has been seen at standard doses of gemcitabine or with other combinations of Chk1 inhibitors with gemcitabine. Tumor responses were observed in anogenital and other solid tumors.

Introduction

DNA damage in cancer cells occurs as a result of multiple endogenous and exogenous factors. Endogenous factors include rapid proliferation caused by oncogenic signaling and inability to repair DNA damage due to defective repair mechanisms or abnormal cell-cycle checkpoints; exogenous factors may include chemotherapy or radiotherapy used in cancer treatment (1). Checkpoint kinase 1 (Chk1) is a key component of the ATR–Chk1–Wee1 pathway; it is activated in response to replication stress (RS) and double-strand DNA breaks and is associated with stability of the cell-cycle S-phase. Cancer cells can have a loss of fidelity of the G_1–S checkpoint and oncogenic signaling, which leads to RS. In this context, the role of Chk1 in cell survival is critical (2). The current study investigated the combination of the novel Chk1 inhibitor SRA737 (Sierra Oncology, Inc.) and low doses of the chemotherapeutic agent gemcitabine. Gemcitabine, a pyrimidine analogue, undergoes a series of phosphorylation steps to be converted to its active form, gemcitabine triphosphate, which is then incorporated into DNA and RNA where it causes DNA damage and RS (3, 4). In addition, gemcitabine is an irreversible inhibitor of ribonucleotide reductase, a critical enzyme responsible for the production of the dNTP, which are important building blocks of DNA replication. Importantly, preclinical work has shown that low, non-cytotoxic concentrations of gemcitabine in combination with Chk1 inhibition can result in tumor growth inhibition, thought to be a consequence of dNTP depletion, resulting in stalled replication forks, RS, and activation of Chk1 (5–7). SRA737 is a novel, orally bioavailable, selective Chk1 inhibitor that has shown activity as a single agent and in combination with gemcitabine in preclinical models (8–10). The combination of SRA737 and a low dose of gemcitabine is hypothesized to have synergistic antitumor activity while circumventing some of the expected toxicities of DNA damage response inhibitors in combination with gemcitabine (11–17).

Patients and Methods

Study design

The objectives of this first-in-human, Phase I/II, open-label, dose-escalation study were to establish the safety profile, recommended

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Translational Relevance

Checkpoint kinase 1 (Chk1) is a key component of the response to replication stress (RS) within DNA and a regulator of the G_2–M cell-cycle checkpoint. This article describes the clinical study of the Chk1 inhibitor SRA737 delivered orally 24 and 48 hours after low-dose gemcitabine (LDG). LDG has low myelotoxicity and causes RS in tumors, allowing unrepaired DNA within S-phase cancer cells past the G_2–M check point leading to cell death. In the expansion phase, patients with genetic alterations related to tumor suppression, DNA damage repair, or oncogenic drivers were enrolled, all of which would cause endogenous RS potentially enhancing response. Of 65 evaluable patients 7 partial tumor responses were observed, including 3 patients with anogenital cancer harboring alterations in FANC/BRCA/PIK3CA, intermediate to high tumor mutational burden, and possibly increased RS from human papillomavirus infection. These partial responses provide proof-of-concept of the efficacy of LDG plus SRA737 that warrants further evaluation.

Phase 2 dose (RP2D), pharmacokinetics (PK) profile, and clinical activity [including objective response rate (ORR) and duration of response] of SRA737 in combination with low-dose gemcitabine (LDG). The trial (ClinicalTrials.gov identifier NCT02797977, EudraCT Number: 2015–004467–36) was conducted at 21 centers in the UK and Spain between August 3, 2016 and April 8, 2020. Research ethics committees approved the study protocol before initiation of patient enrollment, and the study was carried out in accordance with the Declaration of Helsinki, the International Conference on Harmonization Guidelines for Good Clinical Practice, and applicable local regulations. The study was approved in the UK by the Research Ethics Committees (REC) London Center and in Spain by the REC at 12 de Octubre Hospital (Madrid). All patients provided written informed consent before taking part.

Participants

The dose-escalation phase included patients with solid tumors after prior standard-of-care chemotherapy, World Health Organization (WHO) performance status 0–1, and organ function within limits of standard Phase I studies (Supplementary Methods). Tumor type–specific expansion cohorts were planned to recruit up to approximately 20 prospectively identified genetically defined patients in each cohort. Enrollment of expansion cohorts was initiated before the completion of dose escalation with subjects enrolled at the highest dose level determined to be safe and tolerable at the time of their enrollment. Subjects were able to undergo intra-patient dose escalation to receive higher doses of SRA737 and/or gemcitabine if a higher dose level had been deemed safe and tolerable.

The prevalence of genetic alterations related to increased RS hypothesized to enhance response to Chk1 inhibition varies depending on the tumor type. To select for patients with higher levels of endogenous RS, and therefore potentially greater benefit from SRA737 + LDG in the expansion phase, patients were selected with tumor types known to harbor high levels of genomic instability: high-grade serous ovarian cancer (HGSOC), small cell lung cancer (SCLC), soft tissue sarcoma (STS), anogenital cancer, or cervical cancer. In addition, patients with HGSOC or STS were required to have the presence of specific genetic alterations related to tumor suppression, DNA damage repair, replicative stress, or oncogenic drivers. Tumor-specific eligibility criteria for expansion cohorts are summarized in **Table 1**. On the basis of the eligibility criteria of an earlier version of the protocol, patients with urothelial and rectal cancers were also enrolled in the expansion phase.

Table 1. Tumor-specific eligibility requirements for expansion cohorts.

Expansion cohort	Tumor type–specific eligibility requirement
HGSOC	Known germline BRCA mutations or alterations in genes related to tumor suppression, DNA damage repair, replicative stress, or oncogenic drivers (Supplementary Methods)
STS	Alterations in genes related to tumor suppression, DNA damage repair, replicative stress, or oncogenic drivers (Supplementary Methods)
SCLC	Not required to have genetic testing due to the known high incidence of TP53 mutations
Anogenital or cervical cancer	Not required to have genetic testing due to the known high incidence of human papillomavirus (HPV)

This analysis focuses on patients treated with the doublet combination of SRA737 and LDG.

Treatment and dose escalation

A single dose of SRA737 was given at one visit on days –7 to –4 (before the start of cycle 1) for PK assessments. Study treatment was given in 28-day cycles: SRA737 was administered orally on days 2, 3, 9, 10, 16, and 17, and gemcitabine was given intravenously on days 1, 8, and 15 of each cycle. This dosing regimen was based on *in vitro* and *in vivo* preclinical modeling of SRA737 and gemcitabine that demonstrated maximum efficacy when SRA737 was administered 16–24 hours following gemcitabine (10).

Dose escalation of SRA737 in combination with varying doses of gemcitabine was conducted in cohorts of three to six patients according to a rolling-six design wherein once the first subject completed the 7-day observation period following the first dose of gemcitabine, subsequent subjects in that cohort started treatment. Patients were assessed for dose-limiting toxicity from the first SRA737 dose (days –7 to –4) until the end of cycle 1 (up to 35 days). Safety and other supporting data were reviewed by the cohort review committee consisting of the lead investigator, study investigators representing the site(s) currently enrolling patients, and representatives of the study sponsor before dose escalation of SRA737 and/or gemcitabine. A minimum of 3 subjects with no DLT, or 6 subjects with up to 1 DLT were required before escalation to the next SRA737 plus gemcitabine dose level. Dose escalation of SRA737 was started at 40 mg per day and increased in up to 100% increments until the C_{min} of SRA737at 24 hours reached 100 nmol/L. Thereafter, the dose of SRA737 was increased in less than 100% increments (typically 25%–75%). Gemcitabine dose was started at 300 mg/m^2 and could escalate to a maximum of 600 mg/m^2.

Expansion cohorts of up to 20 patients with specified tumor profiles were treated with SRA737 and gemcitabine doses selected by the cohort review committee based on all available safety and PK data; expansion doses were at, or lower than, the MTDs from the dose-escalation phase. Patients could continue treatment until disease progression or discontinuation from the study due to unacceptable toxicity, investigator/sponsor decision, or withdrawal of consent.

Assessments

Safety assessments, including adverse events, laboratory parameters, electrocardiograms, and echocardiograms, were conducted

throughout treatment and until 30 days after the last study treatment or initiation of new anticancer treatment. Toxicity was recorded using National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03. Serial sampling of blood for PK assessment was conducted before and after dosing with single-agent SRA737 (10 time points over 48 hours) and on cycle 1 day 10 following administration of SRA73 and gemcitabine. Plasma SRA737 was quantified using LC/MS (18).

Radiologic tumor assessments were performed every two cycles, and tumors were assessed using the RECIST version 1.1 (19). The ORR was defined as the percentage of patients with a best response of complete response or partial response to treatment according to RECIST criteria. Clinical response data were summarized in cohorts defined by tumor type, including indication-specific expansion cohorts (anogenital, cervical, HGSOC, SCLC, and STS); a grouping of patients with rectal cancer who were enrolled in the dose-escalation phase, and four patients with urothelial cancer enrolled under previous protocol versions.

Statistical analysis

The safety-evaluable population included all patients who received at least one dose of either investigational medicinal product (SRA737 or gemcitabine). The response-evaluable population included patients who had measurable disease at baseline, received at least 83% of planned SRA737 doses in cycle 1, and had at least one postbaseline disease assessment or discontinued treatment due to disease progression, adverse event, or death.

Data availability statement

The trial sponsor, Sierra Oncology, commits to share clinical study data with qualified researchers to enable enhancement of public health. As such, Sierra will share anonymized patient-level data on request or if required by law or regulation. Qualified scientific and medical researchers can request patient-level data for studies of Sierra pharmaceutical substances listed on ClinicalTrials.gov and approved by health authorities in the United States and the EU. Patient-level data for studies of newly approved pharmaceutical substances or indications can be requested 9 months after FDA and European Medicines Agency approvals. Such requests are assessed at Sierra's discretion, and the decisions depend on the scientific merit of the proposed request, data availability, and the purpose of the proposal. If Sierra agrees to share clinical data for research purposes, the applicant is required to sign an agreement for data sharing before data release, to ensure that the patient data are de-identified. In case of any risk of re-identification on anonymized data despite measures to protect patient confidentiality, the data will not be shared. The patients' informed consent will always be respected. If the anonymization process will provide futile data, Sierra will have the right to refuse the request. Sierra will provide access to patient-level clinical trial analysis datasets in a secured environment upon execution of the data sharing agreement. Sierra will also provide the protocol, statistical analysis plan, and the clinical study report synopsis if needed. For additional information or requests for access to Sierra clinical trial data for research purposes, please contact us at: Medinfo@sierraoncology.com.

Results

Patient demographics

A total of 143 patients were enrolled in the SRA737 and LDG treatment cohorts. They included 58 patients across 13 dose-escalation cohorts and 85 patients in the expansion cohorts (**Fig. 1**). In the analysis of tumor response, groups of patients identified by tumor-type were defined (15 with anogenital cancer, 15 with rectal cancer, 12 with cervical cancer, 24 with HGSOC, 22 with SCLC, 11 with STS, and 4 with urothelial cancer). In these groups, a total of 18 patients who participated in dose escalation are included (15 with rectal cancer, 1 with anogenital cancer, 1 with cervical cancer, and 1 with STS). The RP2D was determined to be 500 mg SRA737 combined with low-dose (250 mg/m^2) gemcitabine. Including patients with intra-patient dose escalation, the majority (77 of 143) received SRA737 at doses of at least 500 mg in combination with gemcitabine 250 mg/m^2.

The median age of patients was 62 years (range, 54–68 years), the male/female ratio was 39.2%/60.8%, and WHO performance status 0/1 ratio was 44.1%/55.9% (Supplementary Table S1). HGSOC ($n = 24$), SCLC ($n = 22$), anogenital cancer ($n = 15$), and rectal cancer ($n = 15$) were the most common tumor types. The median number of prior lines of therapy was 2 (range 1 to 9 lines).

Safety profile

The most common treatment-emergent adverse events irrespective of relationship to SRA737 or gemcitabine included nausea (61.5%), vomiting (54.5%), fatigue (51.0%), diarrhea (49.0%), and anemia (45.5%). The incidence of grade ≥3 toxicities was low (**Table 2**).

In a previous study of SRA737 monotherapy in patients with advanced cancer, daily dose (QD) levels from 20 to 1,300 mg were evaluated. The MTD was determined to be 1,000 mg QD with DLTs observed at daily doses of 1,000 to 1,300 mg, including gastrointestinal events, neutropenia, and thrombocytopenia. The RP2D of SRA737 monotherapy was 800 mg QD. At the monotherapy RP2D, common toxicities with SRA737 included diarrhea, nausea, and vomiting that were generally mild to moderate.

The starting dose of SRA737 (40 mg QD) in combination with gemcitabine was chosen to be conservative due to the potential overlapping toxicity with gemcitabine and consideration that with the allowed 100% dose-escalation increments, the 150-mg dose modeled to exceed the minimal effective dose in humans could be reached in a timely manner by the third escalation cohort. The starting dose of 300 mg/m^2 gemcitabine is approximately one third of a typical clinical dose and is based on preclinical models where synergistic antitumor effect of SRA737 plus gemcitabine was observed at gemcitabine doses approximately one third of the typical dose in that model.

Following the enrollment of 13 dose-escalation cohorts (**Fig. 1**), no protocol-defined dose-limiting toxicities had occurred and the cohort review committee determined the MTD was not reached. As described later in this report, the RP2D was declared on the basis of an overall assessment of tolerability in patients alongside preclinical data.

In 60 patients treated with the RP2D, the predominant toxicities were gastrointestinal, with nausea, diarrhea, and vomiting reported by 63.3%, 55.0%, and 56.7% of patients, respectively. Although prophylactic antiemetics or antidiarrheals were not mandated in the study, their use was left to the clinical judgment of the investigators where clinically indicated. The rates of grade ≥3 events for these toxicities were 3.3%, 3.3%, and 6.7%, respectively, and gastrointestinal adverse events led to treatment discontinuation in one patient due to nausea, two patients due to vomiting, and one patient due to diarrhea. The relatively low rate of treatment discontinuation due to gastrointestinal toxicities in comparison with the overall frequency of gastrointestinal events reported suggests that these do not substantially affect the tolerability of SRA737 in combination with gemcitabine, and no special precautions are required. However, appropriate management of gastrointestinal effects, including prophylaxis such as an anti-emetic regimen, would be advised with the SRA737 plus gemcitabine combination where clinically indicated.

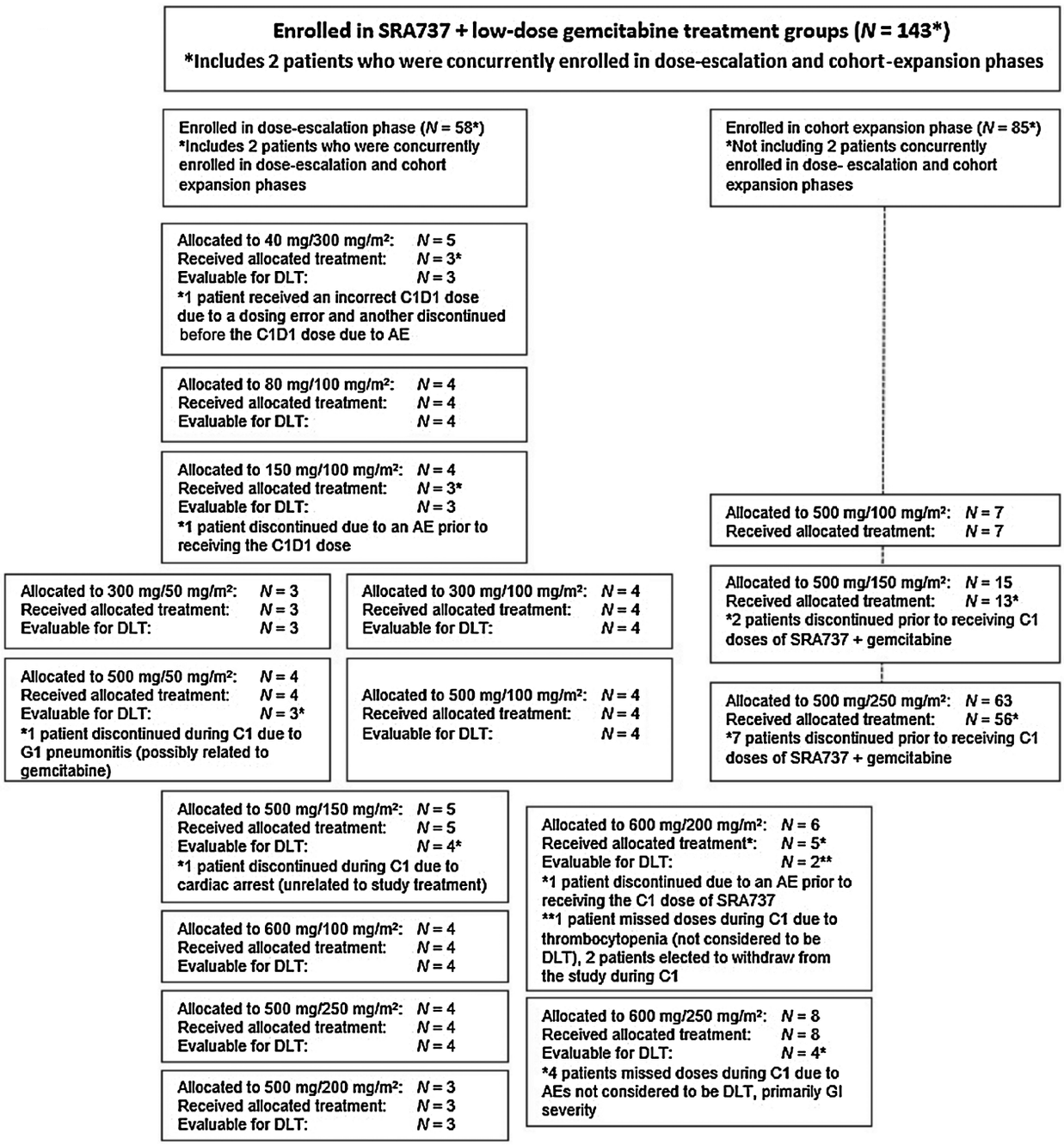


Figure 1.

Enrollment by SRA737 and low-dose gemcitabine dose level. This figure represents the number of patients enrolled at each SRA737 plus low-dose gemcitabine dose level. In addition, the number of patients who received their allocated treatment in each cohort and the number who were evaluable for dose-limiting toxicity in the dose-escalation phase are shown. The SRA737 dose is listed first, followed by gemcitabine dose. Abbreviations: AE, adverse event; C1D1, cycle 1 day 1; DLT, dose-limiting toxicity; G1, grade 1; GI, gastrointestinal.

Other toxicities of note were fatigue (58.3%), anemia (56.7%), neutropenia (46.7%), and thrombocytopenia (41.7%), with grade ≥3 events occurring in 3.3%, 11.7%, 16.7%, and 10.0%, respectively (**Table 3**).

Adverse events leading to treatment discontinuation were reported for 29 (20.3%) patients. The most common event leading to treatment discontinuation was disease progression (3 patients), followed by fatigue, lung infection, metastases to central nervous system, intestinal obstruction, thrombocytopenia, and vomiting (2 patients each); all other reasons for discontinuation applied to only 1 patient each. Events leading to discontinuation that were assessed as causally related to

Table 2. Treatment-emergent adverse events reported by ≥10% of the overall patient population.

Preferred term	SRA737 dose <500 mg (*N* = 30)	SRA737 dose ≥500 mg (*N* = 113)	Overall (*N* = 143)
Any treatment-emergent adverse event	29 (96.7)	113 (100)	142 (99.3)
Nausea	13 (43.3)	75 (66.4)	88 (61.5)
Vomiting	17 (56.7)	61 (54.0)	78 (54.5)
Fatigue	9 (30.0)	64 (56.6)	73 (51.0)
Diarrhea	11 (36.7)	59 (52.2)	70 (49.0)
Anemia	14 (46.7)	51 (45.1)	65 (45.5)
Pyrexia	7 (23.3)	41 (36.3)	48 (33.6)
Thrombocytopenia	8 (26.7)	39 (34.5)	47 (32.9)
Neutropenia	5 (16.7)	44 (38.9)	49 (34.3)
Decreased appetite	4 (13.3)	40 (35.4)	44 (30.8)
ALT increased	7 (23.3)	33 (29.2)	40 (28.0)
AST increased	7 (23.3)	30 (26.5)	37 (25.9)
Constipation	5 (16.7)	30 (26.5)	35 (24.5)
Back pain	8 (26.7)	17 (15.0)	25 (17.5)
Influenza-like illness	5 (16.7)	18 (15.9)	23 (16.1)
Urinary tract infection	4 (13.3)	18 (15.9)	22 (15.4)
Cough	2 (6.7)	19 (16.8)	21 (14.7)
Dyspnea	6 (20.0)	15 (13.3)	21 (14.7)
Abdominal pain	4 (13.3)	16 (14.2)	20 (14.0)
Headache	7 (23.3)	12 (10.6)	19 (13.3)
Asthenia	2 (6.7)	14 (12.4)	16 (11.2)

Note: The terms "thrombocytopenia" and "neutropenia" are inclusive of the terms "platelet count decreased" and "neutrophil count decreased." Patients with multiple adverse events within the same preferred term were only counted once within the respective category. Data are *n* (%) of patients.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 3. Treatment-emergent adverse events reported by ≥10% of patients treated at the RP2D.

	Patients treated at the RP2D of 500 mg SRA737 + 250 mg/m^2 gemcitabine (*N* = 60)		
Preferred term	Grade 1–2	Grade 3–4	All grades
Nausea	36 (60.0)	2 (3.3)	38 (63.3)
Fatigue	33 (55.0)	2 (3.3)	35 (58.3)
Diarrhea	31 (51.7)	2 (3.3)	33 (55.0)
Vomiting	30 (50.0)	4 (6.7)	34 (56.7)
Anemia	27 (45.0)	7 (11.7)	34 (56.7)
Neutropenia	18 (30.0)	10 (16.7)	28 (46.7)
Thrombocytopenia	19 (31.7)	6 (10.0)	25 (41.7)
Pyrexia	23 (38.3)	1 (1.7)	24 (40.0)
Decreased appetite	22 (36.7)	1 (1.7)	23 (38.3)
AST increased	13 (21.7)	3 (5.0)	16 (26.7)
ALT increased	12 (20.0)	3 (5.0)	15 (25.0)
Cough	12 (20.0)	0	12 (20.0)
Urinary tract infection	12 (20.0)	0	12(20.0)
Constipation	11 (18.3)	2 (3.3)	13 (21.7)
Asthenia	10 (16.7)	0	10 (16.7)
Back pain	9 (15.0)	0	9 (15.0)
Dyspnea	9 (15.0)	1 (1.7)	10 (16.7)
Rash	9 (15.0)	0	9 (15.0)
Abdominal pain	8 (13.3)	2 (3.3)	10 (16.7)
Hypomagnesemia	6 (10.0)	1 (1.7)	7 (11.7)
Influenza-like illness	6 (10.0)	0	6 (10.0)
Rash maculopapular	6 (10.0)	0	6 (10.0)
Edema peripheral	5 (8.3)	1 (1.7)	6 (10.0)
Lower respiratory tract infection	2 (3.3)	4 (6.7)	6 (10.0)

Note: The terms "thrombocytopenia" and "neutropenia" are inclusive of the terms "platelet count decreased" and "neutrophil count decreased." Patients with multiple adverse events within the same preferred term were only counted once within the respective category. Data are *n* (%) of patients.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

SRA737 occurred in only 4.9% of subjects, and only two of these related AEs were reported in more than a single subject; fatigue and vomiting occurred in two subjects each. Fatal adverse events were reported for 10 patients (6 were progression of disease, 1 cardiac arrest, 1 lung infection, 1 respiratory failure, and 1 small bowel obstruction); none of these were attributed to SRA737, however, one fatal event of cardiac arrest was considered possibly related to gemcitabine.

Adverse events related to cardiac failure have been recorded in previous Phase I trials (13); cardiac parameters were therefore analyzed in the current study. Of the 143 patients treated with SRA737 and LDG, 80 had baseline and postbaseline (cycle 2 day 1) echocardiograms. Five patients had a ≥10 percentage point absolute reduction in ejection fraction, and of these, four had ejection fraction values of >50% at all time points. One patient's ejection fraction dropped from 60% to 43% but this patient did not exhibit symptoms of cardiac failure. Grade 3 QTcF prolongation (QTcF values of >500 msec and/or increase in QTcF by >60 msec) was seen in seven patients; four of these patients had a maximum QTcF of <500 msec, and none of the QTcF elevations was associated with cardiac signs or symptoms. One patient had cardiac arrest during the study, which was a grade 5 event.

PK profile

The maximum plasma concentration (C_{max}) of SRA737, area under the concentration-time curve from 0 to 12 hours (AUC_{0-12}), half-life, and clearance were measured at SRA737 doses of 40 to 600 mg (**Table 4**). The systemic exposure to SRA737 (AUC_{0-12} and C_{max}) was approximately dose-proportional, particularly at doses within the 150 to 300 mg range (Supplementary Fig. S1).

In preclinical models, synergistic antitumor effect of SRA737 plus LDG has been observed at gemcitabine doses approximately one third of the typical dose in preclinical studies. SRA737 at dose levels of 150 mg or higher result in plasma concentrations modeled from preclinical work to exceed the minimal effective dose in humans. On the basis of this model, the plasma concentrations of SRA737 observed in patients who received SRA737 at dose levels of 150 mg or higher, in combination with LDG, are predicted to produce an antitumor effect, consistent with the efficacy signal observed in this clinical study.

Determination of the RP2D

SRA737 at 500 mg administered 24 and 48 hours following gemcitabine infusion, in combination with gemcitabine at 250 mg/m^2 given on days 1, 8, and 15 of a 28-day cycle, was determined to be the RP2D. This decision was based on overall tolerability, particularly in terms of gastrointestinal and hematological toxicity, which may be associated with SRA737 and gemcitabine (**Table 2**), and a PK profile showing plasma concentrations of SRA737 reaching the minimal effective concentration of SRA737 extrapolated from preclinical models (Supplementary Fig. S2).

Tumor response

Sixty-five patients were treated with SRA737 and LDG and included in the per-protocol response-evaluable population for tumor types of anogenital cancer, cervical cancer, HGSOC, rectal cancer, SCLC, STS,

Table 4. PK parameters for plasma SRA737.

Day	Dose (mg)	t_{max} (h)	C_{max} (ng/mL)	AUC_{0-12} (ng•h/mL)	$t_{1/2}$ (h)	CL (L/h)	V_d (L)
−7 to −4	40	1.8–2.3	61.4–155	—	10.3–17.4	40–75	—
	80	2.0–2.1	11–173	—	10.8–11.9	69–104	—
	150	2 (1–2)	548 ± 63.9	2,630 ± 944	12.7 ± 1.13	38.0 ± 15.9	717 ± 357
	300	2 (1–6)	995 ± 449	4,530 ± 1,590	11.7 ± 1.07	46.0 ± 16.5	764 ± 241
	500	2 (1–8)	1,470 ± 605	8,330 ± 3,390	11.6 ± 2.22	42.3 ± 22.1	695 ± 342
	600	2 (1–4)	1,720 ± 556	10,200 ± 2,970	10.7 ± 2.11	39.1 ± 11.4	597 ± 199
C1D10	40	1.1–2.2	83.3–152	—	—	—	—
	80	1.9–2.2	89.3–142	—	—	—	—
	150	2 (2–2)	478 ± ID	2,390 ± ID	—	—	—
	300	1 (1–4)	1,080 ± 563	5,140 ± 1,610	—	—	—
	500	2 (1–12)	1,580 ± 645	9,410 ± 4,270	—	—	—
C1D10 /C1D17	600	2 (1–6)	1,740 ± 509	9,990 ± 2,920	—	—	—

Note: Data for 40 and 80 mg doses displayed as minimum–maximum; data for 150 to 600 mg doses displayed as median (minimum–maximum) for t_{max} and as median ± SD for other parameters. "—" indicates values that were not calculated. At C1D10 the $t_{1/2}$, CL, and V_d were not assessed due to the shortened PK sampling schedule at this timepoint.

Abbreviations: AUC_{0-12}, area under the concentration-time curve from 0 to 12 hours; C1, cycle 1; CL, total clearance rate of the drug from plasma; C_{max}, maximum plasma concentration; D10, day 10 (of cycle); D17, day 17 (of cycle); h, hour; $t_{1/2}$, elimination half-life; t_{max}, time of maximum plasma concentration; V_d, apparent volume of distribution.

and urothelial cancer. The ORR was 10.8% (7/65) across all cohorts. No complete responses were observed, and 7 patients had a best response of partial response (PR). PRs were seen in anogenital cancer, 3/12 (25%); cervical cancer, 1/6 (16.7%); HGSOC, 1/15 (6.7%); rectal cancer, 1/10 (10%); and SCLC, 1/9 (11.1%; **Fig. 2**). The duration on therapy in patients in the expansion cohort is shown in **Fig. 3**.

Discussion

This is the first clinical report of a Chk1 inhibitor with a novel, low-dose (250 mg/m^2) gemcitabine combination designed to provide exogenous replicative stress while minimizing gemcitabine-associated myelotoxicity and maximizing Chk1 inhibition. It is also the first clinical report of SRA737 used in combination.

Several Chk1 inhibitors have been evaluated in trials with gemcitabine chemotherapy (13, 15–18). However, the lowest dose of gemcitabine recommended for Phase II evaluation was 500 mg/m^2 and the majority of clinical trials proposed that the 1,000 mg/m^2 dose should be used for further study. However, at this standard dose of 1,000 mg/m^2, gemcitabine is known to have significant myelotoxicity. The pharmacological basis of previous single-agent, LDG explored in a clinical setting stems from the knowledge that the rate-limiting enzyme for the activation of gemcitabine, deoxycytidine kinase, is saturated at concentrations of gemcitabine in circulation after infusion at 250 mg/m^2 (17). DNA repair studies now suggest that gemcitabine is a potent inducer of DNA replication fork stress via inhibition of ribonucleotide reductase, activating ATR and Chk1 to allow for DNA repair before mitosis (11, 20, 21). The current study exploits this hypothesis to evaluate LDG (at levels of 50–300 mg/m^2), with the RP2D of gemcitabine in combination with SRA737 being 250 mg/m^2, which is significantly lower than that used in routine clinical practice. The RP2D of SRA737 in the combination was 500 mg for 2 days beginning 24 hours after gemcitabine administration. The plasma SRA737 concentrations achieved at these dose levels were in excess of 40–500 ng/mL, the range corresponding to the minimal effective dose extrapolated from preclinical models. Although the study protocol did include a provision for non-mandatory tumor biopsy analysis to study pharmacodynamic effects, none were obtained and this is a shortcoming of the current study.

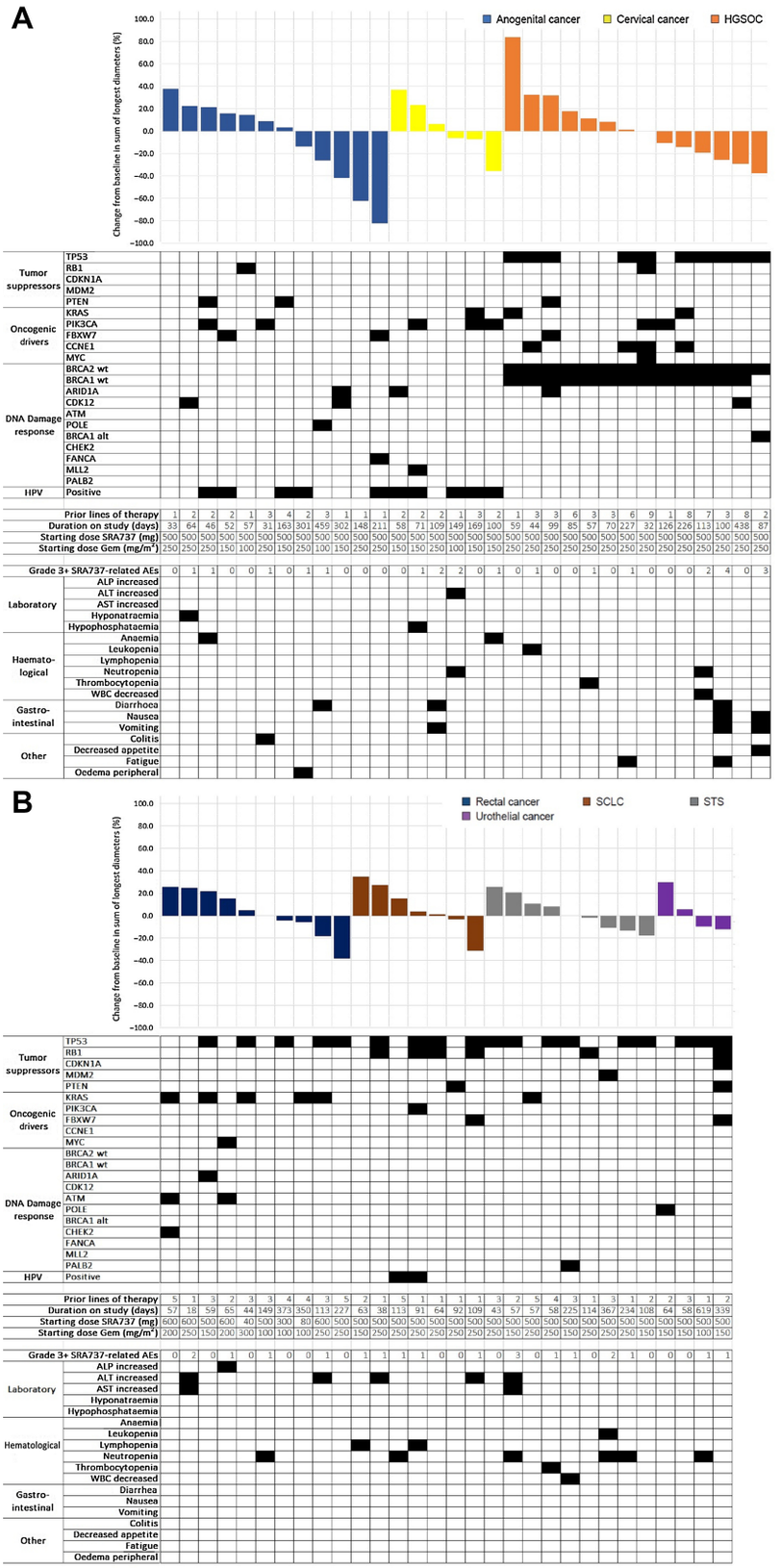
The adverse-effect profile in the current study differs significantly from other gemcitabine and Chk1 inhibitor combinations (11–17). Interestingly, the grade ≥3 neutropenia and thrombocytopenia rates in the current study were 16.7% and 10%, respectively, at the RP2D. These rates of neutropenia and thrombocytopenia are lower than those described at maximally tolerated doses of Chk1 inhibitor and gemcitabine combinations: AZD7762 (71% and 0% at the MTD; ref. 11); GDC-0425 (38% and 12%; ref. 15); and GDC-0575 (79% and 14%; ref. 16). At the RP2D, gastrointestinal side effects of nausea and vomiting occurred in 63.3% and 56.7% of patients, respectively; these were grade ≥3 in 3.3% and 6.7% of patients, respectively. Similar upper gastrointestinal toxicities were seen in other oral Chk1 plus gemcitabine combinations, such as GDC-0425 and GDC-0575, but were less frequent with the intravenous Chk1 inhibitor AZD7762.

There were seven patients with partial responses in the current study: three with anogenital cancer and one each with rectal cancer, HGSOC, SCLC, and cervical cancer. These occurred at gemcitabine dose levels of 250 mg/m^2 or lower. Clinical responses in Chk1 inhibitor and gemcitabine combinations have been seen in patients across a variety of tumor types in Chk1 inhibitor plus gemcitabine combinations: AZD7762 [non–small cell lung cancer (NSCLC); ref. 11], GDC-0425 [ref. 15; triple-negative breast cancer (TNBC), melanoma], and GDC-0575 (TNBC, sarcoma, NSCLC; ref. 16). Of note, the doses of gemcitabine at which these responses were seen were 1,000 mg/m^2 (AZD7762), 750–1,000 mg/m^2 (GDC-0425), and 500 mg/m^2 (GDC-0575); however, it is difficult to analyze the contribution of gemcitabine alone, versus the combination of gemcitabine and Chk1 inhibitors, to these reported responses. There have been no Phase II trials of single-agent full-dose gemcitabine in anal cancers and response rates for full-dose gemcitabine in cervical cancer range from 0% to 11% (22). Given the modest numbers of patients with anogenital cancer (response rate 25%) treated in this study it is difficult to extrapolate if full-dose gemcitabine would have had equal activity to the combination of SRA737 + LDG. Equally, given the low response rate of full-dose gemcitabine, it is unlikely that treatment with gemcitabine alone at the low 250 mg/m^2 dose would have resulted in responses; it is more likely that the combination was effective.

Several of the robust responses observed in this study were associated with genomic alterations in the FA/BRCA network and related

Figure 2.

A–B, SRA737 and low-dose gemcitabine: best tumor response by tumor type. This figure displays the best tumor response per RECIST version 1.1 criteria in the per-protocol response-evaluable population (REP). Prior lines of therapy, starting doses of study treatment, duration on study, and grade 3 or higher AEs related to SRA737 for each patient are also shown. Three patients (1 with HGSOC, 2 with SCLC) included in the REP discontinued before completing a post-treatment tumor assessment and therefore best response could not be determined for these patients. HGSOC, high-grade serous ovarian cancer; SCLC, small cell lung cancer; STS, soft tissue sarcoma.



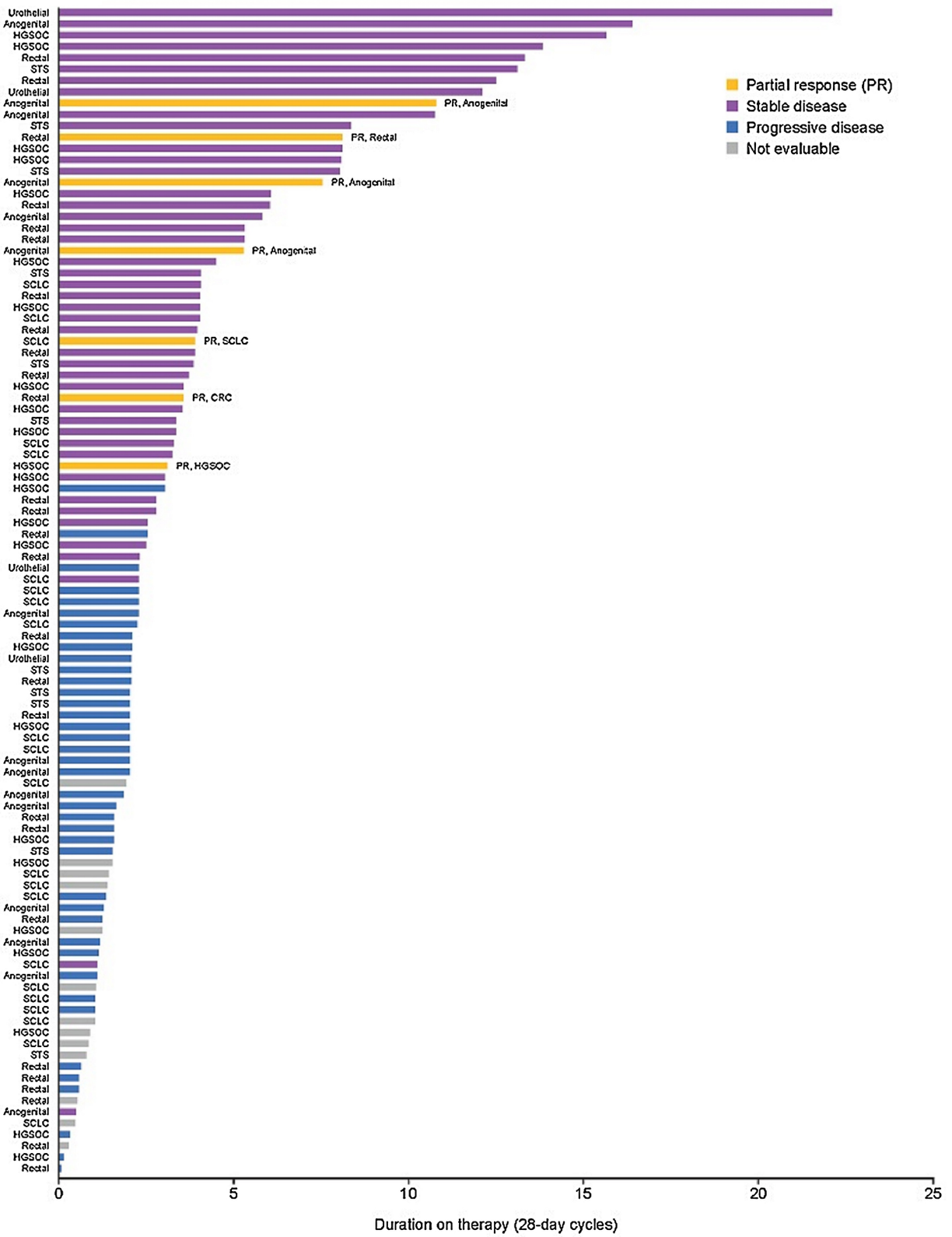


Figure 3.

SRA737 and low-dose gemcitabine: duration on treatment and best response. This figure displays the duration on therapy (cycles) for each patient in the per-protocol response-evaluable population, and their categorical best tumor response per RECIST version 1. HGSOC, high-grade serous ovarian cancer; SCLC, small cell lung cancer; STS, soft tissue sarcoma.

factors involved in replication fork repair. The response in anogenital cancers is noteworthy. Where genetic profiles were available for two of the three responding anogenital tumors, they showed alterations in FANC/BRCA genes or CDK12/ARID1A, and intermediate to high tumor mutational burden. Although it was not possible to confirm HPV infection in all samples, it is conceivable that an HPV infection could cause a functional abrogation of the G_1–S checkpoint, as has been established in preclinical models (21).

The interaction of Chk1 inhibition with immune response has been documented in preclinical models (23, 24) and early clinical trials (25). The combination of SRA737 with LDG plus an immune checkpoint inhibitor has been shown to be effective in SCLC models (26). As it is unlikely there would be overlapping toxicities with combinations of SRA737 and LDG doublets with anti–programmed death-1 antibodies, the addition of anti–programmed death-1 antibodies could increase response rates in tumor types with an unmet need. Given the preclinical data and observations in the expansion cohorts, anogenital tumors and SCLCs are cancers with a significant unmet need for where SRA737 + LDG doublet or a further combination with an immune checkpoint inhibitor as a triplet therapy are of particular interest for further evaluation of SRA737.

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Note

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References

1. Pilie PG, Tang C, Mills GB, Yap TA. State-of-the-art strategies for targeting the DNA damage response in cancer. Nat Rev Clin Oncol 2019;16:81–104.
2. Smith HL, Southgate H, Tweddle DA, Curtin NJ. DNA damage checkpoint kinases in cancer. Expert Rev Mol Med 2020;22:e2.
3. Ciccolini J, Serdjebi C, Peters GJ, Giovannetti E. Pharmacokinetics and pharmacogenetics of gemcitabine as a mainstay in adult and pediatric oncology: an EORTC-PAMM perspective. Cancer Chemother Pharmacol 2016;78:1–12.
4. Smith SC, Petrova AV, Madden MZ, Wang H, Pan Y, Warren MD, et al. A gemcitabine sensitivity screen identifies a role for NEK9 in the replication stress response. Nucleic Acids Res 2014;42:11517–27.
5. Caffo O, Thompson C, De Santis M, Kragelj B, Hamstra DA, Azria D, et al. Concurrent gemcitabine and radiotherapy for the treatment of muscle-invasive bladder cancer: a pooled individual data analysis of eight phase I-II trials. Radiother Oncol 2016;121:193–98.

6. Heinemann V, Xu YZ, Chubb S, Sen A, Hertel LW, Grindey GB, et al. Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2',2'-difluorodeoxycytidine. Mol Pharmacol 1990;38:567–72.
7. Shewach DS, Hahn TM, Chang E, Hertel LW, Lawrence TS. Metabolism of 2',2'-difluoro-2'-deoxycytidine and radiation sensitization of human colon carcinoma cells. Cancer Res 1994;54:3218–23.
8. Lainchbury M, Matthews TP, McHardy T, Boxall KJ, Walton MI, Eve PD, et al. Discovery of 3-alkoxyamino-5-(pyridin-2-ylamino)pyrazine-2-carbonitriles as selective, orally bioavailable CHK1 inhibitors. J Med Chem 2012;55:10229–40.
9. Walton MI, Eve PD, Hayes A, Henley AT, Valenti MR, De Haven Brandon AK, et al. The clinical development candidate CCT245737 is an orally active CHK1 inhibitor with preclinical activity in RAS mutant NSCLC and Emicro-MYC driven B-cell lymphoma. Oncotarget 2016;7:2329–42.
10. Walton MI, Eve PD, Hayes A, Valenti MR, De Haven Brandon AK, Box G, et al. CCT244747 is a novel potent and selective CHK1 inhibitor with oral efficacy alone and in combination with genotoxic anticancer drugs. Clin Cancer Res 2012;18:5650–61.
11. Sausville E, Lorusso P, Carducci M, Carter J, Quinn MF, Malburg L, et al. Phase I dose-escalation study of AZD7762, a checkpoint kinase inhibitor, in combination with gemcitabine in US patients with advanced solid tumors. Cancer Chemother Pharmacol 2014;73:539–49.
12. Hansen RJ, Strouse B, Anderes K, Smith G, Hassig C. The Chk1 inhibitor, SRA737, demonstrates chemical synthetic lethality with replication stress-inducing agents, including low-dose gemcitabine, in preclinical models of cancer. Mol Cancer Ther 2018;17:B181.
13. Seto T, Esaki T, Hirai F, Arita S, Nosaki K, Makiyama A, et al. Phase I, dose-escalation study of AZD7762 alone and in combination with gemcitabine in Japanese patients with advanced solid tumours. Cancer Chemother Pharmacol 2013;72:619–27.
14. Calvo E, Braiteh F, Von Hoff D, McWilliams R, Becerra C, Galsky MD, et al. Phase I study of CHK1 inhibitor LY2603618 in combination with gemcitabine in patients with solid tumors. Oncology 2016;91:251–60.
15. Infante JR, Hollebecque A, Postel-Vinay S, Bauer TM, Blackwood EM, Evangelista M, et al. Phase I study of GDC-0425, a checkpoint kinase 1 inhibitor, in combination with gemcitabine in patients with refractory solid tumors. Clin Cancer Res 2017;23:2423–32.
16. Italiano A, Infante JR, Shapiro GI, Moore KN, LoRusso PM, Hamilton E, et al. Phase I study of the checkpoint kinase 1 inhibitor GDC-0575 in combination with gemcitabine in patients with refractory solid tumors. Ann Oncol 2018;29:1304–11.
17. Zwitter M, Kovac V, Smrdel U, Vrankar M, Zadnik V. Gemcitabine in brief versus prolonged low-dose infusion, both combined with cisplatin, for advanced non–small cell lung cancer: a randomized phase II clinical trial. J Thorac Oncol 2009;4:1148–55.
18. Zangarini M, Berry P, Sludden J, Raynaud FI, Banerji U, Jones P, et al. Development and validation of a LC-MS/MS method for the quantification of the checkpoint kinase 1 inhibitor SRA737 in human plasma. Bioanalysis 2017;9:1001–10.
19. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
20. McNeely S, Conti C, Sheikh T, Patel H, Zabludoff S, Pommier Y, et al. Chk1 inhibition after replicative stress activates a double strand break response mediated by ATM and DNA-dependent protein kinase. Cell Cycle 2010;9:995–1004.
21. Thompson R, Eastman A. The cancer therapeutic potential of Chk1 inhibitors: how mechanistic studies impact on clinical trial design. Br J Clin Pharmacol 2013;76:358–69.
22. Mutch DG, Bloss JD. Gemcitabine in cervical cancer. Gynecol Oncol 2003;90:S8–S15.
23. Chaudhary R, Slebos RJC, Song F, McCleary-Sharpe KP, Masannat J, Tan AC, et al. Effects of checkpoint kinase 1 inhibition by prexasertib on the tumor immune microenvironment of head and neck squamous cell carcinoma. Mol Carcinog 2021;60:138–50.
24. Wayne J, Brooks T, Landras A, Massey AJ. Targeting DNA damage response pathways to activate the STING innate immune signaling pathway in human cancer cells. FEBS J 2021;288:4507–40.
25. Do KT, Manuszak C, Thrash E, Giobbie-Hurder A, Hu J, Kelland S, et al. Immune modulating activity of the CHK1 inhibitor prexasertib and anti–PD-L1 antibody LY3300054 in patients with high-grade serous ovarian cancer and other solid tumors. Cancer Immunol Immunother 2021;70:2991–3000.
26. Sen T, Della Corte CM, Milutinovic S, Cardnell RJ, Diao L, Ramkumar K, et al. Combination treatment of the oral CHK1 inhibitor, SRA737, and low-dose gemcitabine enhances the effect of programmed death ligand 1 blockade by modulating the immune microenvironment in SCLC. J Thorac Oncol 2019;14:2152–63.