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ORIGINAL ARTICLE

A phase I study of capecitabine and a modulatory dose of irinotecan in metastatic breast cancer

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Abstract
Purpose There is a need for chemotherapy regimens active against anthracycline- and taxane-refractory breast cancer. Data from preclinical and pilot studies performed at Roswell Park Cancer Institute (RPCI) suggested that when irinotecan (IRN) is given with 5-fluorouracil (5-FU) efficacy is affected by the sequence of drug administration. Pretreatment with IRN 24 h before 5-FU increased the number of tumor cells in S-phase and the antitumor activity in a preclinical system. These data provided the rationale for the evaluation of IRN and capecitabine, a 5-FU prodrug, sequentially administered in patients with metastatic breast cancer. The main objective of the study was to determine the MTD and identify dose-limiting toxicities (DLTs) of capecitabine and IRN. Additionally, the degree of accumulation of cells in S-phase in tumor biopsies obtained at 24 h after the first dose of IRN was measured in consenting patients.
Patients and methods Metastatic breast cancer patients who experienced disease progression after at least one (taxane or anthracycline based) chemotherapy regimen and an expected survival of at least 3 months and ECOG performance status 0–2 were eligible. Twelve patients were enrolled and treated. The starting doses were IRN 80 mg/m^2 given over 90 min on days 1, 8, 22, 29, and capecitabine 1,500 mg/m^2/day given days 2–15 and 23–36. Evaluation for response was performed after the first cycle. Sequential tumor biopsies were performed on five patients.
Results The first three patients treated exhibited modulation in cyclin A index on tumor biopsy as defined by the study, defining the modulatory dose of IRN as 80 mg/m^2. Overall, 4/5 biopsies showed modulation. Dose Limiting Toxicities (DLTs) were assessed during the first cycle of therapy. Two DLTs (Grade 3 nausea vomiting and dehydration; grade 3 pneumonia, hypoxia, hypotension) were seen at dose level 2 of capecitabine (2,000 mg/m^2/day) and the first cohort was expanded. There were no DLTs for patients treated at DL 1. No grade 3–4 toxicities occurred at DL 1. Seven patients were evaluable for response following one cycle of treatment (partial response 1, stable disease 4, progressive disease 2) Of the five inevaluable patients, two experienced DLT, one received 50% of the planned capecitabine dose, one progressed prior to evaluation, and one withdrew consent.
Conclusion IRN 80 mg/m^2 days 1, 8, 22, 29 in combination with capecitabine 1,500 mg/m^2/day in divided dose days 2–15 and 23–36 has an acceptable toxicity profile and resulted in modulation of S-phase in 4/5 specimens examined. Further studies of the activity of this combination and modulatory effect of IRN are warranted.

Keywords Metastatic breast cancer · Chemotherapy · Irinotecan · Capecitabine · S-phase modulation · Phase I

Introduction

Breast cancer is the most common malignancy in US women, with an annual incidence of newly diagnosed cases exceeding 200,000; more than 40,000 women will die of breast cancer this year despite available therapies [1]. There are a number of drugs with activity in metastatic breast cancer, but the duration of response to the currently available agents tends to be short-lived. The preferred front-line chemotherapy for metastatic breast cancer is typically based on an anthracycline or taxane. Because the majority of patients

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who are treated with anthracycline or taxane-based treatment experience disease progression, there is a need for agents with efficacy against anthracycline and taxane-refractory metastatic breast cancer [2].

5-Fluorouracil (5-FU) has a long history of activity in breast cancer. Bolus 5-FU infusions, with leucovorin, have been shown to have substantial activity, yielding response rates of 36% in chemotherapy naïve patients [3] and 17–23% in patients with prior chemotherapy [4–6]. Continuous infusion 5-FU with leucovorin has yielded response rates of 29% [7]. Although the results associated with continuous infusion 5-FU for metastatic breast cancer are encouraging, this therapy is associated with inconvenience and cost in patient quality of life and medical resources.

Capecitabine (Xeloda®) is a novel, orally administered fluoropyrimidine carbamate used in metastatic breast cancer. Readily absorbed by the gastrointestinal tract, capecitabine is metabolized by the enzyme carboxylesterase in the liver, where it is converted to 5′deoxy-5-fluorocytidine (5′DFCR), which is then converted by the enzyme cytidine deaminase to 5′deoxy-5-fluorouridine (5′DFUR) [8]. In tumor and normal tissues, the enzyme thymidine phosphorylase (TP) converts 5′DFUR to 5-FU. However, unlike parenterally administered 5-FU, oral capecitabine is preferentially activated in tumor tissue and produces higher concentrations of 5-FU in tumor compared with adjacent healthy tissue and plasma [9]. As a result, orally administered capecitabine allows physicians to mimic continuous infusion 5-FU without the complications and costs associated with parenteral therapies and infusion pumps. Capecitabine was approved in 1998 by the US. Food and Drug Administration as a single agent for patients with metastatic breast cancer resistant to both paclitaxel and anthracyclines, and for those with breast cancer resistant to paclitaxel and for whom further anthracycline therapy is contraindicated.

Irinotecan (IRN) (CPT-11, Camptosar®) a camptothecin derivative with increased water solubility, is a prodrug, which is metabolized by carboxylesterase to the active metabolite SN-38 by removal of a bulky dipiperidino side chain. SN-38 targets topoisomerase I (topo 1) an enzyme responsible for the relaxation of supercoiled DNA during replication and transcription. Topo 1 forms a cleavable complex with one strand of duplex DNA, producing a transient single strand break. SN-38 binds to the cleavable complex, stabililizing it and preventing religation of the DNA strand. This leads to single and double strand DNA breaks which are responsible for the antitumor activity. IRN has demonstrated activity (response rate 23%, median response duration 4.9 months) and good tolerability as a single agent in refractory metastatic breast cancer previously treated with taxanes and anthracyclines [2].

Although IRN exhibits antitumor activity in breast cancer at higher (100 mg/m^2 weekly or 240 mg/m^2 every 3 weeks) doses, we have explored in a series of studies, lower dose IRN as a modulator of S-phase to increase the cytotoxic effect of 5-FU. This concept is based on in vitro studies on the combination of 5-FU and IRN or its active metabolite SN-38, which reported superiority of the sequence of IRN followed by 5-FU in a variety of cell lines. Pavillard et al. reported the effect of SN-38 and 5-FU in six cell lines. The sequence of SN-38 followed by 5-FU was the most cytotoxic ($P = 0.0012$). Flow cytometry studies showed a shift of cells from G1 to S-G2 following SN-38 [10]. In studies at Roswell Park Cancer Institute (RPCI) of the Ward colorectal carcinoma model in the rat, synergistic antitumor activity was noted when IRN was given prior to the antimetabolite 5-FU; IRN given 24 h prior to 5-FU resulted in 95% complete tumor regression (CTR) (defined as complete disappearance of the tumor for >90 days), compared to giving the drugs in the reverse order which resulted in a 38% CTR [11]. This was observed with an IRN dose as low as 12.5% of the MTD. These observations suggested a modulatory role for IRN as a sensitizer of tumor cells to 5-FU given 24 h later. Additional experiments were conducted with the HCT-8 cell line in vivo; treatment with IRN resulted in a 152% increase of cells in S-phase, compared to pretreatment with 5-FU, which did not cause an increase. The time course of the effect of IRN on the response of HCT-8 xenografts in nude mice to 5-FU showed a maximum at 24 h post-IRN but a persistence of the effect which was 50% of maximum at 3 days. On the basis of these results, we conducted pilot clinical studies with IRN and the antimetabolites 5-FU and gemcitabine (GEM), both S-phase specific drugs, in patients with advanced, biopsiable solid tumors. In these studies, IRN was administered 24 h before each dose of either 5-FU (400 mg/m^2/week, or GEM (1,000 mg/m^2/week). These studies provided support for the conclusions that (1) pretreatment with IRN produces an increase in the percentage of tumor cells staining for cyclin A (cyclin A index) by immunohistochemistry (IHC); (2) Flow cytometry indicated that the increase is in the S phase than in G2; 3). Eighty mg/m^2 of IRN is more effective than lower doses at producing this modulation [12].

In more recent preclinical studies, the antitumor activity of capecitabine and IRN, administered alone and in combination was evaluated in nude mice bearing human colon tumors HCT-8 and HT-29. Tumor cells were transplanted subcutaneously on day 0 and treatment began when tumor sizes approached 200–250 mg, normally within 7–10 days. Capecitabine was administered orally, daily for 5 days per week × 3 and IRN intravenously, weekly for 3 weeks. Final antitumor assessment was carried out ~2–3 months post-termination of therapy. The results are summarized in Table 1. Given in this way the combination is highly synergistic with minimal toxicity. The doses used are lower than the maximum tolerated dose (MTD) of each drug.

Table 1 Synergistic therapeutic interaction between irinotecan and capecitabine against human colon tumor xenografts (HCT-8 and HT-29, human colorectal adenocarcinoma cell lines): role of drug and schedule

Treatment	Dose	Complete tumor response (%CR)	
		HCT-8 (%)	HT-29 (%)
Irinotecan	50 mg/kg/week × 3	20	0
Capecitabine	400 mg/kg/day 1–5 × 3 weeks	0	0
Irinotecan + capecitabine	50 mg/kg/week × 3 + 400 mg/kg/day 1–5 × 3 weeks	100	20

Administration of the MTDs of both drugs produced severe toxicity with no additional therapeutic benefit [13]. These preclinical and clinical data combined with the known activity of capecitabine in breast cancer prompted a trial to explore the effect of IRN on S-phase in breast tumors and the combination of a modulatory dose of IRN and capecitabine in this disease in a Phase I trial.

The objectives of this Phase I trial were to (1) determine the MTD of capecitabine when capecitabine is given bid days 2–15 and 23–36 and an S-phase modulatory dose of IRN is given on days 1, 8, 22, 29; (2) identify dose-limiting and other major toxicities of the combination given on this schedule; (3) note any antitumor activity.

Patients and methods

This protocol was reviewed and approved by the Institutional Review Board at RPCI and all the patients provided written informed consent prior to entering into this trial.

Eligibility criteria

Patients with metastatic breast cancer who progressed on at least one prior chemotherapy regimen (not including adjuvant therapy) were eligible for study entry. Additional inclusion criteria were as follows: Eastern Cooperative Oncology Group performance score <2 and expected survival >3 months, adequate bone marrow function (absolute neutrophil count $>1.5 \times 10^9$/l, platelet count $>10^{12}$/l, hemoglobin >9.0 g/dl); and adequate renal (serum creatinine <1.5× ULN or creatinine clearance >50 ml/min) and hepatic function (serum bilirubin <1.5× ULN, AST <2× ULN). Patients of childbearing potential were required to use appropriate contraception. Patients could not have had chemotherapy in the previous 3 weeks or major surgery or radiation in the prior 4 weeks (except small port radiotherapy for symptom control). Patients were excluded for uncontrolled infection or other uncontrolled medical illness. Patients who had tumor accessible to biopsy were asked to consent for serial biopsies.

Pretreatment evaluation

Before study admission, all patients underwent a complete history and physical examination, chest X-ray, assessment of performance status, CT scan of the chest, abdomen and pelvis and a bone scan (if indicated). A CBC with differential and serum chemistry, as well as pregnancy test in patients of childbearing potential were obtained within one week of study entry. Weekly blood counts and serum chemistries were obtained on treatment, and physical examination, and radiologic assessment of tumor response repeated every 6 weeks.

Treatment of patients

Chemotherapy

Irinotecan was given on days 1, 8, 22, 29 (starting dose 80 mg/m^2); capecitabine was given on days 2–15 and 23–36 (starting dose 750 mg/m^2/bid). All patients received standard antiemetic prophylaxis and were given prescriptions for antidiarrheals to use as required.

Drug was held for grade IV hematologic toxicity and grade III non-hematologic toxicity (except controllable nausea and vomiting) according to the National Cancer Institute Common Toxicity Criteria (Version 2.0). Chemotherapy was resumed at a 33% dose reduction when toxicity reduced to grade 0. Grade III hematologic toxicity or grade II non-hematologic toxicity resulted in dose delay until the toxicity was reduced to grade 0–1, without subsequent dose reduction.

Tumor biopsies

Core biopsies of tumor tissue were performed in consenting patients with accessible tumor before and 24 h after the first dose of IRN for measurements of the percentage of cells in S-phase. The biopsies were sent through tissue procurement and pathology to the laboratory of Dr. Rustum for evaluation. S-phase was measured by IHC for cyclin A and where possible by DNA flow cytometry.

Immunohistochemistry (IHC) for cyclin A

Antigen retrieval was achieved with citrate buffer (pH 6.0) in a microwave for 2 × 10 min before casein blocking. Sections were incubated with primary antibody (mouse antihuman cyclin A, 0.1 μg/ml Novacastra laboratories, Newcastle, UK), overnight at 4°C and with secondary antibodies from Vecastein Universal Elite ABC Peroxidase Kit (Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions. Slides were incubated with chromogen containing H_2O_2 for 10 min at room temperature. Negative controls were isotype matched mouse IgG1 at the same concentration as the primary antibody. Human tonsil was used as known positive (germinal centers) and known negative (lymphocytes) control. The pre- and post-biopsies on a patient were prepared together. In biopsies where the amount of tumor tissue permitted, 300–400 tumor nuclei were read. Where fewer than this number was present, all of the tumor nuclei were read. The cyclin A index was the number of positive cells divided by the total number of tumor cells evaluated [11].

DNA flow cytometry

Biopsy samples for FC were placed in ice cold Hanks Balanced Salt solution and taken to the Flow Cytometry laboratory. They were disaggregated and divided into three aliquots. These were centrifuged at 3,200 rpm at 4°C for 3 min, decanted and vortexed. In preparation for data acquisition, aliquot I was stained by the addition of 5 μl of 5 μg/ml ethidium monoazide, exposed to UV light (350 nm) for 15 min, washed with PBS and fixed with 2% formaldehyde. Aliquot II was analyzed unstained, for comparison, for calculation of percentage of dead cells.

Aliquot III was fixed in 70% ethanol for a minimum of 30 min at 4°C, centrifuged as above, decanted and vortexed, 1 ml of 0.5% BSA added and again centrifuged as above. After decanting and vortexing, 1 ml of 0.05 mg/ml propidium iodide was added and staining carried out for a minimum of 30 min at 4°C. Data acquisition was on a FAC scan (BD Biosciences, Palo Alto, CA, USA) and analysis was carried out using the programs Winlist and Modfit (Verity Software House, Topsham, ME, USA) and Multicycle (Phoenix Flow Systems Inc., Castasauqua, PA, USA).

Study design, definitions, and end points

The primary objective of the study was to determine the MTD of capecitabine given twice daily on days 2–15 and 23–36 with IRN given i.v. on days 1, 8, 22, and 29 at a modulatory dose, arbitrarily defined as a dose giving at least a 50% increase in S-phase in tumor tissue, 24 h after drug administration in at least two-thirds of the patients treated. S-phase was assayed by IHC for cyclin A and confirmed where possible by flow cytometry. In studies with other patient populations, IRN at 80 mg/m^2/week had been established as the modulatory dose [12].

The study was carried out in two stages. In the first stage, IRN 80 mg/m^2 was evaluated with pre- and post-treatment biopsies to confirm this dose as a modulatory dose in this patient population. The dose of capecitabine was 750 mg/m^2 bid. In the second stage this dose of IRN was kept constant and the dose of capecitabine was escalated to the MTD of the combination.

Determination of MTD

A standard 3 × 3 design was used in which three patients are initially entered at the first dose level (DL1). If there is no DLT, three patients are entered at DL2. This is continued until one or more patients show a DLT. If one patient has a DLT, three more patients are added, but at that DL; if no further DLT is seen, escalation continues. If ≥2 DLT are seen at a dose level, escalation stops and the previous dose level is expanded to six patients. If this dose level has 0 or 1 DLT it is the MTD. If it has ≥2 the dose is again reduced until the highest dose with <1 DLT is defined. This is the MTD. There is no intra patient dose escalation.

Results

Patients

Twelve patients were entered onto this single-center trial. All patients had metastatic breast cancer with progression on at least one prior regimen in the metastatic setting. The median number of prior regimens was three (range 1–7).

Sequential tumor biopsies were performed on five patients. Patients continued on study drug until disease progression or unacceptable toxicity. Evaluation for response and toxicities was performed after one cycle (6 weeks) and every 6 weeks thereafter.

Dose escalation and DLTs

Three patients were evaluable for toxicity on dose level one (IRN 80 mg/m^2 days 1, 8, 22, 29 and capecitabine 1,500 mg/m^2/day in divided dose). An additional two patients were entered on DL 1 but withdrew consent prior to evaluation. The first three patients treated exhibited modulation in cyclin A index on tumor biopsy as defined by the study, establishing the modulatory dose of IRN in this population as 80 mg/m^2. Overall, 4/5 biopsies exhibited modulation. There were no DLTs at dose level one.

Escalation progressed to DL 2 (IRN 80 mg/m^2 days 1, 8, 22, 29 and capecitabine 2,000 mg/m^2/day). Three patients were treated at this dose; there were two DLTs. One patient developed hypoxia and hypotension due to pneumonia and required a hospitalization, the other developed grade III nausea and vomiting requiring hospitalization and hydration.

Consequently, an additional three patients were entered at DL 1. A further patient was entered but was not evaluable for toxicity. There were no DLTs at dose level one, and therefore IRN 80 mg/m^2 days 1, 8, 22, 29 and capecitabine 1,500 mg/m^2/day was determined as the MTD.

Safety and adverse events

Table 2 lists all adverse events separately for the nine patients treated on dose level one and the three patients treated on DL 2. No grade 4 events were observed and two patients were hospitalized for treatment-related adverse events.

Hematologic adverse events were generally mild at both dose levels. Four patients developed a grade II anemia, and three patients a grade II neutropenia. Granulocyte colony-stimulating factors were not administered prophylactically during the study. No grade 3–4 toxicities were observed at DL 1.

Table 2 Dose Limiting Toxicities (DLTs) were assessed during the first cycle of therapy using the NCI CTC Version 2.0

Toxicity	Grade 1	Grade 2	Grade 3
Fatigue	1	2	1
Anorexia		2	1
Nausea		1	1
Vomiting	1	1	1
Dehydration		2	
Diarrhea	2	2	
Hypotension		1	1
Neutropenia		3	
Anemia		4	
Hand-foot syndrome		1	
Fever		1	
Hypoxia			1
Pneumonia			1
Hyponatremia			1
Hypokalemia			1
Hypoalbuminemia		1	
Elevated AST	1		

Two DLTs (grade 3 nausea, vomiting, and dehydration; grade 3 pneumonia, hypoxia, and hypotension) were seen at dose level (DL) two of capecitabine (1,000 mg/m^2 bid) and the first cohort was expanded. There were no DLTs for patients treated at DL 1. No Grade 3–4 toxicities were seen at DL 1

Gastrointestinal events were observed more frequently with two patients developing grade II diarrhea and two patients developing Grade I diarrhea. There were no Grade III toxicities at DL 1. At dose level 2 (80 mg/m^2 of IRN and 1,000 mg/m^2/bid capecitabine) two of the three treated patients experienced DLT. One patient required an admission to the hospital for pneumonia with associated hypoxia and hypotension, considered drug related. She recovered with supportive care and IV antibiotics. The second patient with DLT reported grade III anorexia, nausea and vomiting despite antiemetics, grade III dehydration, fatigue, and Grade II diarrhea. She was admitted to the hospital for supportive care and recovered. The six patients who received a full cycle of treatment on DL 1 did not suffer any grade 3 toxicities, and no dose reductions or dose delays were required.

Anti-tumor activity

There were no complete responses noted in this study. Seven patients were evaluable for response to treatment. After one cycle of therapy, one patient exhibited a partial response. Four patients had stable disease and two patients had progressive disease.

S-phase modulation

The original study design included pre- and post-IRN tumor biopsies on all enrolled patients. The study was subsequently amended to expedite patient accrual once the modulatory dose of IRN had been defined in this patient population. The first three patients treated exhibited modulation in cyclin A index on tumor biopsy as defined by the study, establishing 80 mg/m^2 as the modulatory dose of IRN. Overall, 4/5 biopsies showed modulation (Table 3).

Table 3 Tumoral increase in cyclin A and S-phase 24 h post-irinotecan therapy in patients with metastatic breast cancer

Patient number	Cyclin A by IHC			DNA flow cytometry		
	Pre (%)	Post (%)	Increase (%)	Pre (%)	Post (%)	Increase (%)
1	11	24	118	–	25	–
2	6	16	160	4.4	14.8	236
3	8	17	113			
4	21	33	57			
5	24	27	12	30	27	−10

The first three patients treated exhibited modulation in cyclin A index on tumor biopsy as defined by the study, establishing the modulatory dose of irinotecan as 80 mg/m^2 . Overall, 4/5 biopsies showed modulation

Discussion

This Phase I study was conducted to determine the MTD of the combination of capecitabine and a modulatory dose of IRN in patients with metastatic breast cancer. The patients who participated in this trial were heavily pretreated, with the median number of previous chemotherapy regimens reported as three. In this group of MBC patients with refractory disease the combination of IRN and capecitabine at dose level one had a favorable safety profile and was associated with S-phase modulation in 4/5 tumor biopsy specimens.

In preclinical studies, IRN increased the activity of 5-FU in the Ward colon carcinoma model at doses well below the MTD and without an increase in toxicity when administered 24 h prior to 5-FU. Preclinical data suggest that the basis for this observation might be an accumulation of tumor cells in S-phase 24 h after IRN administration. Two pilot trials have been conducted at RPCI to attempt to validate these findings clinically [12]. A study combining IRN with 5-FU was designed as a pilot study to determine the lowest dose of IRN, which would give a consistent increase in S-phase in tumor tissue without increased toxicity. This study was performed in patients with advanced solid tumors, and all patients were required to have two tumor biopsies, one prior to and one following IRN administration. The starting dose of IRN was 80 mg/m^2 given weekly and followed by 400 mg/m^2 of 5-FU. All four patients whose tumor biopsies were evaluable for S-phase met the criteria for S-phase modulation. A dose of 40 mg/m^2 of IRN with 400 mg/m^2 of 5-FU was then evaluated. This dose failed to meet the criteria for modulation. An intermediate dose of 60 mg/m^2 had borderline activity. This established 80 mg/m^2 as the minimum dose to meet the criteria for modulation. At this dose, the median increase in cyclin A index was 120% with a range of 95–200% in four patients. In the two patients on whose samples it was possible to carry out flow cytometry the increases in S-phase were 129 and 264%, and in both cases there was a decrease in G-2, making it highly unlikely that the observed increase in cyclin A index was due to accumulation of cells in G-2. In the present study it was not considered appropriate to explore doses of IRN lower than 80 mg/m^2 as it was unlikely to be in the best interests of the patient.

The present study confirms in breast cancer the potential for synergy between IRN and capecitabine through the increase in the number of tumor cells in S-phase induced by the former, potentially increasing their vulnerability to the fluoropyriminidine antimetabolite. This combined with the known activity of both drugs in MBC and the mode of action of IRN which is different from that of the standard drugs for this disease, make this combination and schedule attractive for further development in breast cancer. It should be noted that, although IRN was used primarily as a modulator in the present study, the dose used (80 mg/m^2/week) was only 20% less than that used in the Phase II studies of IRN as a single agent [2].

Irinotecan has a different mechanism of action from that of other frequently utilized agents in the treatment of metastatic breast cancer. This distinctive mechanism and its tolerability and efficacy as a single agent in other published trials in metastatic breast cancer make it an attractive agent for further study.

The strong topoisomerase I inhibitory activity of IRN and its in vitro synergy with 5-FU in breast cancer cells lines make the combination of IRN and capecitabine an attractive one for Phase II trial investigation in this disease.

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