Clinical Cancer Research

Phase Ib Pilot Study to Evaluate Reparixin in Combination with Weekly Paclitaxel in Patients with HER-2–Negative Metastatic Breast Cancer

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Abstract

Purpose: Chemokine receptor 1 (CXCR1) is recognized as an actionable receptor selectively expressed by breast cancer stem cells (BCSCs). Reparixin is an investigational allosteric inhibitor of chemokine receptors 1 and 2 (CXCR1/2), and demonstrates activity against BCSCs in human breast cancer xenografts. This phase Ib clinical trial examined dose, safety, and pharmacokinetics of paclitaxel plus reparixin therapy, and explored effects of reparixin on BCSCs in patients with metastatic breast cancer (MBC) (trial registration ID: NCT02001974).

Experimental Design: Eligible patients had MBC and were candidates for paclitaxel therapy. Study treatment included a 3-day run-in with reparixin oral tablets three times a day, followed by paclitaxel 80 mg/m^2/week (days 1, 8, and 15 for 28-day cycle) + reparixin tablets three times a day for 21/28 days; three dose cohorts were examined in a 3+3 dose escalation schema. Additional patients were recruited into an expansion cohort at the recommended phase II dose to further explore pharmacokinetics, safety, and biological effects of the combination therapy.

Results: There were neither G4–5 adverse events nor serious adverse events related to study therapy and no interactions between reparixin and paclitaxel to influence their respective pharmacokinetic profiles. A 30% response rate was recorded, with durable responses >12 months in two patients. Exploratory biomarker analysis was inconclusive for therapy effect on BCSCs.

Conclusions: Weekly paclitaxel plus reparixin in MBC appeared to be safe and tolerable, with demonstrated responses in the enrolled population. Dose level 3, 1200 mg orally three times a day, was selected for further study in a randomized phase II trial (NCT02370238). *Clin Cancer Res; 23(18); 5358–65. ©2017 AACR.*

Introduction

Breast cancer stem cells and the CXCL8/CXCR1 axis

Breast cancer stem cells (BCSCs) have the ability to self-renew and generate the full range of cells that make up a bulk tumor (1). Although the ideal marker for BCSCs has not yet been elucidated, the BCSC population is most frequently characterized by the expression and/or activity of the enzyme aldehyde dehydrogenase (ALDH), or by the phenotype CD24$^-$/CD44$^+$ (2). ALDH$^+$ BCSCs account for a small proportion of cells that constitute a bulk tumor, estimated in some studies to comprise between 0.1% and 10% of the bulk tumor (3, 4), so elimination of these cells alone would not result in a sizeable tumor regression in the short term. However, BCSCs display chemoresistance in animal models (5–7) and in patients (8, 9), and it is theorized that the elimination of BCSCs would reduce the risk of cancer progression due to tumor regrowth after treatment. A combination strategy that targets both the chemosensitive bulk tumor and the BCSCs has the potential to improve patient outcomes.

Chemokine receptor 1 (CXCR1) is almost exclusively expressed in the ALDH$^+$ BCSC population compared with its expression in bulk tumor cells (4, 10). CXCR1 is a receptor for CXC ligand 8 (CXCL8; formerly IL8), a proinflammatory chemokine implicated in the metastasis and progression of multiple malignancies, including breast cancers. CXCL8 has also been shown to stimulate self-renewal of BCSCs *in vitro* (4). Tissue damage induced by chemotherapeutic agents may induce CXCL8 as part of the injury response (6, 7). This suggests that strategies aimed at interfering with the CXCL8/CXCR1 axis, activated by conventional chemotherapy (CT), may be able to target BCSCs and increase the efficacy of treatment.

Rationale for reparixin

Reparixin (DF1681Y, formerly repertaxin) is a specific inhibitor of CXCL8 biological activity. Studies to elucidate its mechanism of action have shown that reparixin is a noncompetitive allosteric inhibitor of the CXCL8 receptors CXCR1 and CXCR2. Interaction of reparixin with CXCL8 receptors inhibits the

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Experimental models and retrospective clinical observations point to cancer stem cells (CSCs) as responsible for treatment resistance, disease recurrence, and metastasis. CXCR1, one of the receptors for CXCL8, was identified on breast cancer stem cells (BCSCs) and may serve as an actionable target on these cells. Reparixin, an investigational allosteric inhibitor of CXCR1, reduced the metastatic spread of human breast cancer cells and the BCSC content of human breast cancer xenografts in mice. This article reports the results of a phase Ib study of escalating doses of reparixin oral tablets in combination with weekly paclitaxel in women with HER2-negative metastatic breast cancer. The response rate, median TTP, and the manageable toxicity profile observed support further development of the combination, which is currently being evaluated in first-line metastatic triple-negative breast cancer.

intracellular signal transduction events activated by binding of CXCL8 to CXCR1 and CXCR2 (11, 12). In human breast cancer xenografts, reparixin administered every 12 hours reduced the $ALDH^+$ cell population and the $CD24^-/CD44^+$ BCSC populations when alone or in combination with taxane CT, and reduced formation of metastases following intracardiac injection of tumor cells (6).

At the time of development of the clinical trial, reparixin had been evaluated as an intravenous infusion with good safety and tolerability profile. The starting dose (i.e., 400 mg three times a day) for this trial was selected to reach pharmacologically active plasma concentrations and exposure achieved in recipients of pancreatic islet transplantation (13) while affording a safety factor >5 based upon toxicology studies conducted in rats by the oral route. The three times a day schedule day 1 to 21 with paclitaxel on days 1, 8, and 15 was selected to mirror combination murine efficacy experiments (6). This is the first clinical trial with reparixin oral tablets.

Materials and Methods

A phase Ib clinical trial to examine the dose, safety, and pharmacokinetics of paclitaxel plus reparixin therapy, and to explore the effects of reparixin on BCSCs and the tumor microenvironment, was initiated in patients with metastatic breast cancer (MBC) who were eligible for paclitaxel CT (NCT02001974). The trial was conducted in accordance with the Declaration of Helsinki and the U.S. Common Rule, and was approved by the Institutional Review Boards of each institution. Written informed consent was obtained from all subjects.

Patient population

Subjects were females ≥18 years of age with HER-2 negative MBC. Patients were not known to be refractory to paclitaxel, and if they had previously received paclitaxel in the adjuvant setting, they must have been free of disease progression for >12 months following completion of treatment. If the patient had previous taxane for metastatic treatment, no disease progression may have occurred during taxane treatment or within 3 months of the end of treatment. Prior treatment was limited to no more than three lines of cytotoxic CT in the metastatic setting. Patients had measurable disease according to RECIST criteria version 1.1, ECOG PS ≤1, adequate organ function, and absence of brain metastases and leptomeningeal disease.

Study treatment

Patients were enrolled in cohorts of three to six patients to receive escalating doses of reparixin oral tablets three times a day in combination with a fixed dose of weekly paclitaxel, for as long as clinical benefit was observed. In all cohorts, paclitaxel 80 mg/m^2 was administered on days 1, 8, and 15 of a 28-day cycle. Cohorts 1 to 3 received reparixin 400, 800, and 1200 mg three times a day respectively, on days 1 to 21 of each 28-day cycle. In cycle 1 only, patients received a 3-day course of reparixin alone (day 3 to day 1) at the assigned dose for the cohort, for the purpose of obtaining pharmacokinetic data.

Standard premedications to reduce the incidence of hypersensitivity reactions to paclitaxel were allowed, as were all medications deemed necessary for the supportive care and safety of the patient, including hematopoietic growth factors. The only exception was the use of ibuprofen, which was disallowed due to the slight metabolism of reparixin to ibuprofen. The co-administration of any other anticancer agent or investigational drug was not permitted.

Pharmacokinetic sampling

Pharmacokinetic sampling was performed for cycle 1 only, in the first 19 patients (the pharmacokinetic population). Venous blood samples (6 mL) were collected from a forearm vein at times 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, and 8.0 hours following reparixin administration on days −3 and 21. Day 1 and 8 sampling were similar but omitted the 1.5-, 3.0-, and 6.0-hour time points, and added a collection at 24 hours. The blood samples were immediately centrifuged at 4°C, 1,200 relative centrifugal force (RCF), for 10 minutes, the supernatant cells discarded, and the plasma collected. Each plasma sample was divided into two aliquots and transferred to polypropylene tubes, then stored frozen at −20°C or below until analysis. Analysis was performed at the Dompé Quality Control Department in L'Aquila, Italy.

Efficacy evaluation

Efficacy was evaluated using CT scans, bone scans, and other assessments as required by RECIST 1.1 criteria for response and progression assessment. Each individual site was responsible for response and progression assessment; central review was not performed.

Correlate assays

Peripheral blood samples were obtained for enumeration of CTCs using the FDA-approved CELLSEARCH CTC test (13). Time points included pretreatment day −3, cycle 1 day 15, and at the time of first tumor assessment (cycle 3 day 1). Blood for CTC enumeration was collected using 8.5 mL CellSave tubes and shipped ambient the same day of collection to Fox Chase Cancer Center (Philadelphia, PA). In addition to CTC detection by CELLSEARCH, isolation of CTCs by the exploratory AdnaTest Breast was performed in the first 15 patients as previously reported (14, 15).

EMT-inducing transcription factors (EMT-TFs) were assayed in leukocyte-poor peripheral blood mononuclear cells (LP-PBMCs) in the first 15 patients. Briefly, after depleting CTC with an epithelial phenotype with AdnaBreastSelect, the residual leukocytes were incubated with magnetic beads coated with anti-CD45 antibody to deplete $CD45^+$ leukocytes, as previously described (16, 17). Thereafter, mRNA was extracted from the leukocyte-poor fraction, reversed transcribed to cDNA, and subjected to fluorescence-based qRT-PCR for the detection of EMT-TF gene transcripts, including FOXC, TG, Slug, Snail, Twist, and ZEB. Overexpression of EMT-TFs was defined as at least twice the expression observed in the CD45-depleted fraction, as previously reported (16).

In addition, LP-PBMC samples were interrogated for ALDH activity using the ALDEFLUOR assay and the manufacturer's protocol (StemCell Technologies). For analysis, epithelial cells were defined as cells exhibiting the phenotype $CD326^+CD45$dim. Within the $ALDEFLUOR^+$ epithelial cell population, a subset of CSCs was defined as cells with a $CD326^+CD44^+CD24$ lo phenotype as previously reported (18, 19).

Cytokine inflammation markers (IL1β, IL6, IL8, TNFα, and granulocyte-macrophage colony-stimulating factor) were measured prior to dosing on days −3, 1, 8, and 15 of cycle 1, and day 1 of each subsequent cycle. 7.5 mL of blood was collected in EDTA tubes and immediately placed on ice. The blood samples were immediately centrifuged at 4°C, 2,000 RCF for 10 minutes; plasma was separated into two cryovials and frozen at −70°C until shipped for analysis. Analysis was performed by the Translational Research Laboratory, University of Michigan. A Luminex 200 analyzer was used to detect and quantify the cytokines with a human cytokine 5-plex (Invitrogen).

Dose escalation methods and study endpoint definitions

The primary objective of the study was to evaluate the safety and describe the pharmacokinetic profile of orally administered reparixin in combination with paclitaxel in patients with HER-2 negative MBC and to establish a recommended phase II dose of the combination therapy. Treatment was administered as described above. During dose escalation, a safety assessment was performed after each dose level, and included a review of both safety and pharmacokinetic data from cycle 1 for all patients in that cohort. The outcome of the safety review determined whether patients would be recruited into the next dose cohort.

Dose-limiting toxicity (DLT) was defined as a toxicity that occurred during cycle 1 that was considered possibly, probably, or definitely treatment related. The following events were considered DLTs: fever $\geq$ grade 2; grade 3 liver function tests (LFT) that persisted for over 14 days; in the case of subjects with elevated baseline LFTs due to liver metastases, DLT was defined as an increase in AST/ALT of greater than three times the baseline value or an increase in alkaline phosphatases of greater than twice the baseline value that persisted for over 14 days; grade 3 or 4 gastrointestinal toxicity; grade 3 or 4 neurological toxicity; any other grade 3 or greater nonhematologic toxicity which could be attributable to reparixin; any grade 4 hematologic toxicity which could be attributable to reparixin; or failure to commence the second cycle as scheduled due to lack of recovery to baseline conditions from study drug related toxicities.

Monitoring of treatment emergent adverse events (TEAE) was performed throughout the study and at the off-treatment visit for descriptive presentation in the study results. The sample size was expanded at the recommended phase II dose in order to obtain more data on safety, to perform ECG recordings at the maximum concentration after the first dose and at steady state, to obtain additional data on pharmacokinetics and exploratory biomarkers. The sample size for this study was not based on formal statistical computation.

Assessments of efficacy were secondary objectives. No formal statistical analysis was planned to assess the tumor response data. The disease response was assessed every 8 weeks until the end of the study based on the RECIST version 1.1 criteria. The endpoints included the complete response (CR) rate, partial response (PR) rate, stable disease (SD) rate, progressive disease (PD) rate, median time to progression (TTP), clinical benefit rate (CBR), and the measure of the 6-month progression-free survival rate (%). Patients discontinued, lost to follow up, or dead were censored at the date of last tumor assessment. In the event that no tumor assessment was conducted after the first administration of study drug, day 1 served as the censoring date instead.

Results

Description of enrolled population

This study was opened to accrual on February 15, 2012, closed on December 19, 2014, and included five participating U.S. sites.

Thirty-three patients were enrolled in the study. Three enrolled patients are not included in subsequent analyses for the following reasons: two withdrew consent prior to receiving any dose of study medication and one was discovered to have $Her2^+$ gastric metastases (which made her ineligible) and was removed from study prior to receiving any study medication.

Patient characteristics are summarized in Table 1. As per eligibility, all enrolled patients were female, and the median age of those enrolled was 53.5 years. Eighty-three percent of patients had visceral disease, and the majority had two or more sites of metastasis. Twenty of 33 patients had received prior (neo)adjuvant CT, and 16 of these patients had received a taxane in the adjuvant setting. Nineteen of 33 had received CT in the metastatic setting, with 11 having one prior metastatic regimen and eight having two or more CT regimens. Overall, 13 patients had never received a taxane before enrolling into this trial.

Safety

The safety population included all patients who had taken at least one dose of study drug, $N = 30$; four patients were in cohort 1, three in cohort 2 and 23 in cohort 3. There were no DLTs in any cohort. The most common treatment emergent adverse events (TEAEs) were (all cohorts): fatigue (76.7% of patients), alopecia (63.3%), nausea (60%), constipation (53.3%), decreased appetite (50%), diarrhea (40%), dyspnea (40%), peripheral edema (33.3%), and vomiting (33.3%). TEAE related to study treatment are displayed in Table 2. Grade 3 treatment-related TEAE were rare, representing only 2.7% of all reports. The few grade 3 TEAEs were only observed in cohort 3, likely due to the small numbers of patients treated in cohorts 1 and 2. 79.8% of all ADR reports were grade 1, and 17.4% grade 2. The most frequent TEAEs were gastrointestinal disorders (39.2% of all reports), all grade $\leq$2. Only one patient was reported to experience grade 3 peripheral neuropathy despite several patients exceeding the cumulative dose of

Table 1. Patient characteristics ($N = 30$)

	N (%)
Enrolled	33
Safety population	30
Age, years – median (range)	53.5 (35–80)
<65	22 (73.3)
≥65<75	7 (23.3)
≥75	1 (3.3)
Race	
White	24 (80)
Black	5 (16.7)
Asian	1 (3.3)
ECOG PS	
0	16 (53.3)
1	14 (46.6)
Visceral disease	25 (83.3)
No. of metastatic sites	
1	10 (33.3)
2	15 (50.0)
≥3	5 (16.6)
Prior (neo)adjuvant CT	
None	12 (40.0)
Any regimen	18 (66.0)
Taxane	14 (46.6)
Prior therapy for metastatic disease	
None	8 (26.6)
CT	16 (53.3)
HT	14 (46.6)
CT/HT	8 (26.6)
No. of prior metastatic CT regimens	
0	14 (46.6)
1	9 (30.0)
≥2	7 (23.3)
Prior taxane	
(neo)adjuvant setting	14 (46.6)
(neo)adjuvant + advanced	17 (56.7)

Abbreviations: HT, hormonal therapy; PS, performance status.

1,000 mg/m^2 paclitaxel on study. Five patients experienced a TEAE leading to discontinuation, one in cohort 1 (paclitaxel hypersensitivity reaction) and four in cohort 3: paclitaxel hypersensitivity reaction (one patient), thrombocytopenia related to disease progression (one patient), secondary primary gastric cancer discovered while on therapy (one patient) and grade 2 gastrointestinal disorder following two cycles of therapy (one patient). There was no apparent dose effect of increasing reparixin dose on the incidence, severity or profile of TEAEs experienced by the treatment groups, and there were no clinically significant differences between the treatment groups with regards to laboratory measurements, vital signs, ECG, and physical examination assessments. Seven patients experienced a serious TEAE but none was considered related to reparixin.

Pharmacokinetics

The pharmacokinetic population included all patients who received at least 1 dose of reparixin and had at least one valid, quantifiable pharmacokinetic parameter, $N = 19$. Four patients were in cohort 1, three in cohort 2, and 12 in cohort 3.

Over the range of 400 to 1,200 mg (5.7 to 17.1 mg/kg for a 70 kg subject), reparixin C_{max} and $AUC_{0\text{-}\infty}$ increased as dose increased, in a manner that was approximately dose proportional (Fig. 1A). There appeared to be little or no accumulation of reparixin over the 21-day dose period. After 4 days of three times a day dosing, within the variability observed, concentration of reparixin at each dose level appeared to reach a constant value, which is evidence that steady state may have been reached. Reparixin was rapidly absorbed after oral administration (Supplementary Table S1). $t_{1/2}$ was about 2 hours and was independent of dose level or cycle day (Supplementary Table S1). Likewise, CL/F (oral clearance) and Vz/F (apparent volume of distribution following oral administration) were also independent of dose level or cycle day with mean values of 5.4 L/h and 15 L, respectively. Reparixin was metabolized to the hydroxylated (DF2243Y), carboxylated (DF2188Y), and hydrolyzed (ibuprofen) metabolites (data not shown).

Paclitaxel infusion on day 1 and day 8 started after the day's first dose of reparixin, and the following dose of reparixin was administered after 8 hours. Pharmacokinetic curves of paclitaxel concentrations over time on day 1 and 8 of cycle 1 are shown in Fig. 1B for the three cohorts. Paclitaxel levels were consistent in each treatment group and on each treatment day. t_{max}, C_{max}, and $AUC_{0\text{-}inf}$ were each similar for all cohorts on both cycle days (Supplementary Table S2). Mean $t_{1/2}$ was about 8 hours and was independent of cohort and cycle day (Supplementary Table S2). The above-described pharmacokinetic parameters are comparable with what was previously reported for single agent weekly paclitaxel 80 mg/m^2 (20, 21). There was little or no accumulation from day 1 to day 8. Thus, when administered according to the dosage and schedule evaluated in this study, increasing doses of reparixin oral tablets do not affect the pharmacokinetics of paclitaxel administered as a 1-hour intravenous infusion.

Description of efficacy results

The efficacy population included 27 patients (three in cohort 1, three in cohort 2, and 21 in cohort 3). Three patients in the safety cohort were not included in the efficacy cohort: two had a hypersensitivity reaction to paclitaxel during cycle 1 and then received other CT prior to first disease re-evaluation, one patient withdrew consent before completing cycle 1 and

Table 2. Treatment-related adverse events (with at least 10% incidence in safety population)

ADR	Cohort 1 (*N* = 4), *n* (%)	Cohort 2 (*N* = 3), *n* (%)	Cohort 3 (*N* = 23), *n* (%)	Total (*N* = 30), *n* (%)
Nausea	1 (25)	2 (66.6)	9 (39.1)	12 (40)
Dyspepsia	0	2 (66.6)	7 (30.4)	9 (30)
Vomiting	3 (75)	2 (66.6)	3 (13)	8 (26.7)
Constipation	0	2 (66.6)	3 (13)	5 (16.7)
Fatigue	2 (50)	3 (100)	12 (52.2)	17 (56.7)
Edema, peripheral	0	0	6 (26.1)	6 (20)
Decreased appetite	2 (50)	1 (33.3)	5 (21.7)	8 (26.7)
Cough	1 (25)	0	3 (13)	4 (13.3)
Neutrophil count decreased	0	0	3 (13)	3 (10)
Weight decreased	0	1 (33.3)	2 (8.6)	3 (10)

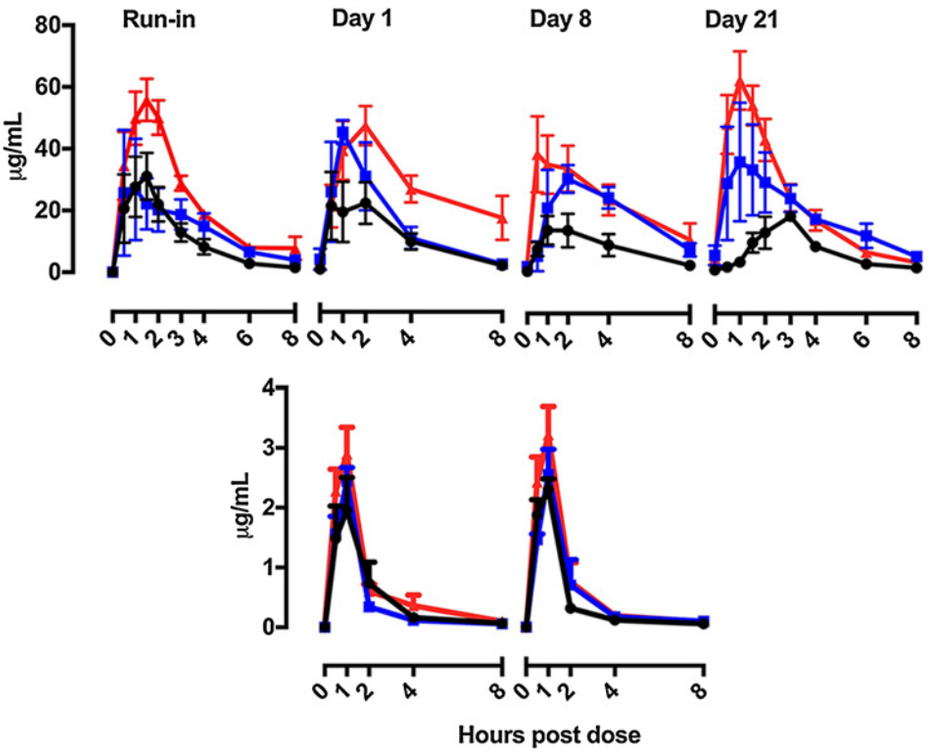

Figure 1.
Top: Mean plasma concentration versus time for reparixin on day −3 (run-in), 1, 8, and 21 of cycle 1. Bottom: Mean plasma concentration versus time for paclitaxel on day 1 and 8 of cycle 1. Black line = cohort 1; blue line = cohort 2; red line = cohort 3.

prior to disease re-evaluation. In total, 8/27 patients had a confirmed RECIST response (29.6%). The majority of responses (6/8) were recorded in patients who had previously been treated with a taxane (Table 3). Of responding patients, all but one were from cohort 3. Median TTP (95% confidence interval) for the three cohorts were 58 days (44–infinity) for cohort 1, 67 days (58–82) for cohort 2 and 162 days (60–229) for cohort 3 (Table 3).

Two patients on study had durable complete responses. One CR patient presented with $ER^-/PR^-/HER2^-$ MBC after having received prior anthracycline and taxane therapy for recurrent breast cancer. This patient was treated on dose level 1. The other CR patient had $ER^+/PR^+/HER2^-$ MBC that relapsed 10 years after adjuvant anthracycline/taxane and endocrine therapy for primary breast cancer, and was treated on dose level 3. Both patients had baseline metastatic disease in lymph nodes and soft tissue only, which is a known to be associated with better prognosis (22). Both CR patients remained in CR at the time of manuscript submission, >36 months after enrollment The ER^- patient received no additional local or maintenance therapies. The ER^+ patient received no additional local therapies and is receiving endocrine therapy. (Anne F. Schott, personal communication). There were no pharmacokinetic or pharmacodynamic parameters that predicted duration of response.

Biomarker studies

A total of 26/27 patients in the efficacy cohort had evaluation of baseline CTCs by CellSearch, and 8/26 had baseline elevation of CTCs defined as >5 tumor cells/7.5 mL blood (Table 4). All eight patients with elevated CTC at baseline had a re-evaluation of CTC at least one other time point, and 2/8 had a reduction of CTC to <5 at any later time point (Table 4). Because of small numbers, no attempt was made to correlate CTC response with clinical outcome. The exploratory CTC isolation utilizing AdnaTest Breast in the first 15 patients identified very few cells, and the results appeared to have little correlation with CellSearch (Supplementary Table S3); thus, no further analysis was performed. In addition, exploratory studies of EMT-TF in LP-PBMC did not reveal any specific patterns of FOXC, TG, Slug, Snail, Twist, ZEB (Supplementary Table S3).

Table 3. Summary of antitumor activity

	Cohort 1 ($N = 3$)	Cohort 2 ($N = 3$)	Cohort 3 ($N = 21$)	Total
ORR	1 (33.3%)	0	7 (33.3%)	8/27 (29.6%)
CR	1 (33.3%)	0	1 (4.7%)	2/27 (7.4%)
PR	0	0	6	6/27 (22.2%)
ORR prior taxane	1/1	0	5/7	6/8
SD >24 weeks	0	0	2	2/27 (7.4%)
CBR	1 (33.3)	0	12 (57)	13/27 (48.1%)
6 months PFS%	1 (33.3)	0	10 (47.6)	11/27 (40.7%)
mTTP days (95% CI)	58 (44–inf)	67 (58–82)	162 (60–229)	

NOTE: CBR, percentage of the patients having 4-month duration of CR, PR, or SD according to RECIST 1.1.

Table 4. CTC count by CellSearch

	Cohort 1 ($N = 3$), *n* (%)	Cohort 2 ($N = 3$), *n* (%)	Cohort 3 ($N = 21$), *n* (%)	Total ($N = 27$), *n* (%)
Baseline CTC <5 [min–max]	2 (66.6)	2 (66.6)	14 (66.6) [0–5]	19 (70.4)
Baseline CTC ≥5 [min/max]	1 (33.3)	1 (33.3)	6 (28.6) [6–699]	8 (29.6)
CTC responder[a]	0	1 (100)	1 (16.7)	2 (25)
CTC reduction[b]	3 (100)	3 (100)	15/21 (71.4)	21/27 (77.7)
mTTP baseline CTC <5 [95% CI]	—	—	162 [58–229]	—
mTTP baseline CTC >5 [95% CI]	—	—	115.5 [1–318]	—

[a]CTC <5 at any time point.
[b]CTC reduction from baseline at any time point.

For the ALDEFLUOR assay on LP-PBMC, 4/18 and 6/18 evaluated patients from cohort 3 were positive at baseline for ALDEFLUOR$^+$/CD326$^+$/CD45$^-$ and ALDEFLUOR$^+$/CD326$^+$/CD24$^-$/CD44$^+$ cells, respectively.

There was high variability in baseline values for cytokines. Serial samples had diminishing patient numbers over time, and in cohorts 1 (400 mg) and 2 (800 mg) there were only one and zero patients after cycle 3, respectively, and these are not further described. In cohort 3, samples were obtained on 21, 20, 13, 12, 11, and 11 patients on cycles 1 to 6, and later cycles are not presented due to diminishing numbers of patients who received more than six cycles of therapy. There was no discernible pattern of change in cytokines over six cycles. Although serum IL8 levels appeared to decline over time with reparixin and paclitaxel (Fig. 2), interpretation of this finding is confounded by many factors, including small numbers of evaluable patients at later time points, high variability in individual patients, and decreasing tumor burden in patients on therapy for longer periods of time.

Selection of recommended phase II dose

Based on observed safety and pharmacokinetic profile, with achievement in all cohorts of plasma concentrations and exposure in the range of recipients of pancreatic islet transplantation (23) with a less than dose proportional increase with increasing dose levels, cohort 3 was selected for further study. This dose level does not represent reparixin maximum tolerated dose.

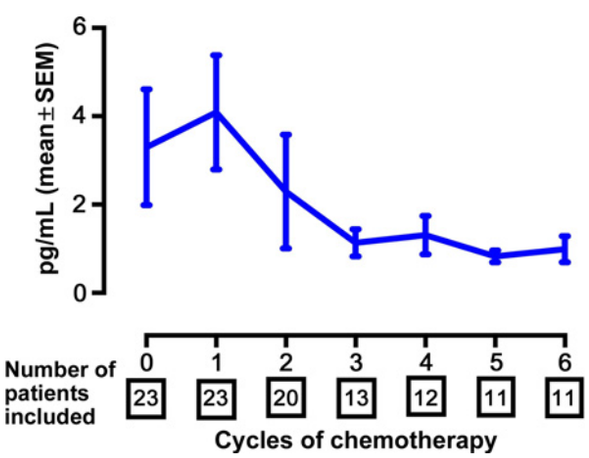

Figure 2.
Plasma concentration over time of IL8 measured on day −3 of cycle 1 and on day 1 of cycles 1 to 6. Results are presented as mean ± SEM. The number of patients evaluated at each time point is indicated.

Discussion

MBC remains an incurable illness in the vast majority of cases, with only occasional exceptions. Although endocrine therapies, cytotoxic chemotherapies, and newer targeted agents may cause reductions in tumor bulk, development of therapy resistance is expected. BCSCs compose a population of cancer cells that are therapy resistant and that can initiate new tumors. It is hypothesized that the eradication of the BCSC population is necessary to achieve the elusive goal of cure of MBC.

Over the last few years, independent laboratories provided preclinical evidence of a role of IL8 in BCSC survival, and demonstrated that blocking CXCR1 or CXCL8 results in anti-CSC activity. This phase Ib trial tested reparixin, an inhibitor of CXCL8 biological activity, in combination with paclitaxel. Our results demonstrate that the addition of reparixin to paclitaxel does not appear to introduce significant additional toxicities (24), even at the highest dose level of reparixin tested, and for prolonged periods of time in several patients. Inhibition of CXCR2 has been reported to cause dose-dependent reductions in absolute neutrophil count and decreased neutrophil tissue responses. We observed neither an increased rate of neutropenia nor of infections in patients receiving weekly paclitaxel compared to historical cohorts, although this would need to be evaluated further in a randomized study setting. Interestingly, a low incidence and severity of grades 3 and 4 peripheral neuropathy was observed compared with published studies with weekly paclitaxel (25). A possible role of reparixin in reducing this common adverse reaction to paclitaxel is being investigated in experimental models (26).

Demonstration of the therapeutic elimination of BCSCs in the clinical setting is desired but thus far has remained elusive (27). Particularly in the metastatic setting, estimation of changes in the proportion of BCSCs in serial tumor samples is hindered both by the rarity of patients from whom at least 2 representative biopsies can be obtained, and the fact that BCSCs constitute a minute population of cells within a bulk tumor. Reduction in BCSCs following single-agent reparixin treatment in serial biopsies has been investigated in a window of opportunity trial in operable HER2$^-$ BC patients (28). In this trial, blood-based biomarkers of BCSCs and on-target action of reparixin were explored. The circulating biomarkers included CTC enumeration by CellSearch and AdnaTest, LP-PMBC-based evaluation of ALDEFLUOR and EMT-TF, and serum cytokine measurements. Unfortunately, no clear pattern of change in any of these markers was observed. This is likely related to multiple issues, including but not limited to small

sample size, low CTC number in the enrolled patient population leading to limited tumor material for testing, and high baseline heterogeneity in the measurements. Although published clinical data in patients with advanced breast cancer indicate that taxane therapy increases serum IL6, GM-CSF, IFNγ levels, and decreases IL1 and TNF (29), we did not observe these patterns in the serum of our patient population over time, and results varied considerably even within individual patients.

Based on this experience, we do not recommend use of these circulating markers for monitoring the effect of anti-CSC agents, and point out a need for development of alternative endpoints in clinical trials of anti-CSC agents, particularly in the metastatic setting. One potential surrogate endpoint is the development of metastases at new sites (30, 31). It is hypothesized that a successful anti-CSC treatment will have an impact on the time to developing new metastases and on the proportion of patients progressing with new metastases rather than on the progression of pre-existing metastases which is likely due to non-BCSC, bulk tumor cells. Interestingly, it has been shown that a combination of reparixin and paclitaxel reduces breast cancer brain metastases formation in mice (32).

In summary, the trial was successful in identifying a safe and tolerated dose of reparixin with favorable pharmacokinetics for further evaluation. The correlative studies, which were conducted in peripheral blood due to unavailability of tumor tissue, were inadequate to demonstrate an effect of the combination treatment on BCSCs, a secondary objective of this trial. We observed a 29.6% confirmed RECIST response rate in a pretreated population of patients with MBC, with two durable complete responders. In ECOG 2100, the overall RECIST response rate for weekly paclitaxel in previously untreated MBC was similar to this trial at 25% (24), despite the fact that the E2100 population was less heavily pretreated and used a slightly higher paclitaxel dose (E2100 utilized 90 mg/m^2 versus the 80 mg/m^2 used in this trial). Based on the hypothesized mechanism of action of reparixin against the rare cancer stem cell population, the study design did not aim to demonstrate a higher objective response rate from the addition of a reparixin to paclitaxel. Efficacy results are presented to support the postulation that there would be no detriment to the expected efficacy of paclitaxel before proceeding to a larger trial. The question of whether an agent that acts on a small subpopulation of cancer stem cells can improve patient outcomes when given in combination with a known active agent such as paclitaxel can only be answered in the context of a randomized clinical trial. Based on the observed safety and preliminary efficacy of the combination therapy in this phase Ib study, a randomized clinical trial of weekly paclitaxel with and without reparixin in first-line treatment of metastatic triple negative breast cancer, with progression-free survival as the primary endpoint and new metastases as an exploratory endpoint, is underway to answer the efficacy question (33).

Disclosure of Potential Conflicts of Interest

M. Cristofanilli is a consultant/advisory board member for Agendia, Dompe, and Vortex. No potential conflicts of interest were disclosed by the other authors.

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