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Phase II evaluation of dasatinib in the treatment of recurrent or persistent epithelial ovarian or primary peritoneal carcinoma: a Gynecologic Oncology Group study.

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ABSTRACT

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Objective: Preclinical data suggest an important role for the sarcoma proto-oncogene tyrosine kinase (SRC) in the oncogenesis of epithelial ovarian cancer (EOC) or primary peritoneal carcinoma (PPC). The Gynecologic Oncology Group (GOG) conducted a Phase II trial to evaluate the efficacy and safety of dasatinib, an oral SRC-family inhibitor in EOC/PPC and explored biomarkers for possible association with clinical outcome.

Methods: Eligible women had measurable, recurrent or persistent EOC/PPC and had received one or two prior regimens which must have contained a platinum and a taxane. Patients were treated with 100 mg orally daily of dasatinib continuously until progression of disease or adverse effects prevented further treatment. Primary endpoints were progression-free survival (PFS) ≥ 6 months and response rate. Serial plasma samples were assayed for multiple biomarkers. Circulating free DNA was quantified as were circulating tumor and endothelial cells.

Results: Thirty-five (35) patients were enrolled in a two-stage sequential design. Of the 34 eligible and evaluable patients, 20.6% (90% confidence interval: 10.1%, 35.2%) had a PFS ≥ 6 months; there were no objective responses. Grade 3-4 toxicities were gastrointestinal (mostly nausea and emesis; n=4), pulmonary (dyspnea and/or pleural effusion; n=4) and pain (n=5), and infrequent instances of anemia, malaise, insomnia, rash, and central nervous system hemorrhage. Lack of clinical activity limited any correlation of biomarkers with outcome.

Conclusion: Dasatinib has minimal activity as a single-agent in patients with recurrent EOC/PPC.

22

INTRODUCTION

23 Despite initially high remission rates, at least 75% of women diagnosed with advanced
24 stage epithelial ovarian carcinoma (EOC) will relapse and ultimately die of their disease [1].
25 Treatment of these patients once they develop recurrent disease remains a major problem. The
26 need for new therapeutic strategies is evident. The SRC family of kinases (SFK) is a nine
27 member group of membrane associated non-receptor tyrosine kinases that are involved in a
28 variety of cellular signaling pathways [2]. The SFK is involved in the oncogenesis of numerous
29 tumors including ovarian cancer. SRC regulates many intracellular signaling pathways
30 responsible for various important tumor cell functions such as proliferation, motility and invasion,
31 angiogenesis, and survival. SRC has been found to be overexpressed in a majority of late
32 stage ovarian tumors and cell lines [3].

33 SRC is a component of signaling pathways downstream of many growth factor
34 receptors, including epidermal growth factor receptor (EGFR), vascular endothelial growth factor
35 receptor (VEGFR), and MNNG transforming gene product (c-MET) [4]. Increased resistance to
36 traditional chemotherapy is modulated by SRC through increased activity of RAS and AKT [5].
37 Inhibition of SRC enhanced the activity of cytotoxic agents, including cisplatin, gemcitabine, and
38 paclitaxel through the activation of caspase-3 in pre-clinical models [5-7]. In addition, tumor
39 growth was blunted when human ovarian cancer cells carrying an antisense SRC construct
40 were implanted into mice bearing these xenografts [8].

41 VEGF is an important growth factor for ovarian cancer cells [9]. VEGF was significantly
42 down-regulated by SRC inhibition and microvessel density was reduced [10]. Anti-VEGF
43 therapy has demonstrated activity in patients with recurrent and primary disease [11,12]. VEGF
44 stimulation of its receptor increased tyrosine phosphorylation of focal adhesion kinase, p130
45 CAS and paxillin [13]. Increased activity of these mediators leads to an increased epithelial-to-

46 mesenchymal transition (EMT), a crucial step to enhancing the metastatic potential of these
47 cancer cells [14]. SRC is a key intermediate in the EMT process [15,16]. In addition, caveolin-1
48 expression indirectly promotes cell-cell adhesion in ovarian cancer cells [17,18]. SRC interferes
49 with caveolin function also promoting EMT and encouraging tumor spread.

50 Dasatinib is a potent oral inhibitor of breakpoint cluster region-Abelson fusion protein
51 (BCR-ABL), c-KIT, ephrin type-A receptor 2 (EPHA2), c-FMS, and SFK [19,20]. These kinases
52 are implicated in oncogenic process and maintaining the metastatic phenotype of many
53 cancers. Dasatinib's mechanism of action depends on its successfully competing for the ATP
54 binding site contained in the kinase domain. The agent is widely approved for chronic
55 myelogenous leukemia and Ph+ acute lymphoblastic leukemia and is now under investigation
56 for treating various solid tumors [21].

57 Based on these observations, the evaluation of dasatinib in patients with recurrent EOC
58 was undertaken by the Gynecologic Oncology Group (GOG). Translational research objectives
59 were included to explore the association between biomarkers and patient outcome. Biomarkers
60 included cell-free DNA (cfDNA), circulating tumor cells (CTCs), circulating endothelial cells
61 (CECs), circulating endothelial precursors (CEPs), and seven plasma biomarkers relevant to
62 dasatinib treatment (EGF and its soluble receptor [sEGFR], VEGF and its soluble receptors
63 [sVEGFR1, sVEGFR2, sVEGFR3], and insulin like growth factor binding protein 2 [IGFBP2]).

64

PATIENTS AND METHODS

65 Eligibility

66 Eligible patients had a histologically-confirmed diagnosis of EOC or primary peritoneal
67 carcinoma, measurable disease as defined by Response Evaluation Criteria in Solid Tumors
68 (RECIST) [22], a GOG performance status of 0-2, and adequate bone marrow (absolute
69 neutrophil count $\geq 1,500/\mu\text{L}$, platelet count $\geq 100,000/\mu\text{L}$), renal (serum creatinine ≤ 1.5 x the
70 upper limit of normal), and hepatic function (total bilirubin ≤ 1.5 x the upper limit of normal, and
71 transaminases and alkaline phosphatase ≤ 2.5 x the upper limit of normal). No biomarker based
72 method was used for patient selection. Eligible patients were permitted to have up to two prior
73 cytotoxic regimens, but if a patient had only one prior regimen, she was required to have a
74 platinum-free interval of less than 12 months or to have progressed during or have persistent
75 disease after platinum-based therapy. Prior biological agents were permitted other than those
76 known to inhibit SRC. Patients with prior radiation to more than 25% of marrow bearing areas,
77 therapeutic warfarin treatment, or signs and/or symptoms of bowel obstruction were excluded.
78 Patients provided written informed consent consistent with federal, state, and local institutional
79 review board at each participating GOG institution in accordance with assurances filed with and
80 approved by the Department of Health and Human Services.

81 Treatment Plan and Dose Modifications

82 Dasatinib (Bristol-Myers Squibb, New York, NY) was administered orally at an initial
83 dose of 100 mg once daily until disease progression or adverse effects required interruption,
84 reduction or discontinuation of therapy. Dose level -1 was 70 mg daily and dose level +1 was
85 70 mg twice daily. Although dosing was continuous, a cycle was defined as 28 days. Dasatinib
86 was supplied by Bristol-Myers Squibb, Inc.

87 Toxicity was graded using the National Cancer Institute Common Toxicity Criteria
88 version 3.0 (NCI-CTCAE v3.0). For first occurrence of febrile neutropenia and/or documented
89 grade 4 neutropenia, dasatinib was held until the absolute neutrophil count (ANC) was grade ≤ 2
90 then reduced by one dose level. Patients with grade 4 thrombocytopenia had their drug held
91 until grade ≤ 1 and then were reduced by one dose level. The next cycle of dasatinib did not
92 begin until the ANC was $\geq 1,500/\mu l$ and the platelet count was $\geq 100,000/\mu l$. Therapy was
93 allowed to be delayed up to a maximum of two weeks. Patients who failed to recover adequate
94 counts within this time were removed from study. Prophylactic use of myeloid growth factors
95 was prohibited. Patients who experienced grade ≥ 2 non-hematologic toxicity had therapy held
96 until resolution to grade 0-1 up to a maximum of 14 days. Dasatinib was then restarted at 70
97 mg daily. If toxicity recurred to grade 2 or worse, the patient would discontinue study drug.
98 Exceptions to the above modifications included: liver function tests were required to be grade 3
99 or worse toxicity before dose modification was required. There was no dose adjustment for
100 fatigue or alopecia. Doses were reduced for gastrointestinal toxicities only if they could not be
101 controlled with medical management. Fluid retention (pleural effusion and/or ascites) was
102 managed with early initiation of diuretic treatment (furosemide and/or spironolactone. Cavity
103 drainage was performed as clinically indicated. Once a patient's dose was reduced, no
104 subsequent increases were permitted.

105 Patients with no grade ≥ 1 toxicities after cycle 1 were escalated one dose level (70 mg
106 twice daily) beginning with cycle 2 of treatment. This dose escalation was included based on
107 early data that solid tumors may require higher doses of dasatinib to achieve clinical activity
108 compared with the doses used to treat patients with CML [23,24].

109 Response Assessment

110 Patients were evaluated clinically every four weeks and radiographically every eight
111 weeks. The same evaluation modality was used throughout for each patient on study.
112 Response criteria used were as defined by RECIST [22].

113

114 Translational Research

115 Plasma and whole blood specimens were collected for translational research prior to cycles 1, 2
116 and 3. Detailed methodology and references for isolating and phenotyping CTC and CEC/CEP
117 can be found in the supplemental material [online only]. Methods for determining circulating
118 levels of serum biomarkers and extraction and quantification of total plasma cell-free DNA
119 (cfDNA) are also summarized in the supplemental online material.

120 Statistical Methods

121 We anticipated that the effect of dasatinib might be either cytotoxic or cytostatic.
122 Therefore, the primary endpoint of this study included both objective tumor response and the
123 proportion of patients alive and progression-free after six months PFS-6). Time on study was
124 assessed from date of registration and included all eligible treated patients.

125 The null hypothesis, i.e. an “uninteresting” level of efficacy, was determined from
126 analysis of an historical GOG dataset, based on a similar patient population from clinical trials of
127 study drugs now considered inactive or minimally active. The null hypothesis jointly specified
128 the probability of a patient experiencing a tumor response to be $\leq 10\%$ and the probability of a
129 patient being alive and free from PFS-6 to be $\leq 15\%$. For the purpose of study design, a 20%
130 increase (to 25% for tumor response or to 35% for PFS-6) were considered clinically significant.
131 The two-stage, bivariate, flexible method of Sill and Yothers [25] was used with a goal of limiting
132 patient exposure to inactive agents while restricting the probabilities of type I and type II errors

133 to about 10%. If the regimen were to demonstrate sufficient activity in the first stage (with 35
134 patients, this required ≥ 5 objective tumor responses or ≥ 8 patients with PFS-6), then the study
135 would target a total of 53 patients (cumulatively) in stage 2. Signed-rank tests were used to test
136 changes from baseline in biomarkers, and Wilcoxon rank sum tests were used to compare
137 changes or ratios for patients who had PFS for at least 6 months versus those who did not.
138 Proportional hazards models were used to compare PFS by high (\geq median) versus low
139 ($<$ median) baseline (pre-cycle 1) levels of each parameter; both unadjusted models and models
140 adjusted for age and performance status were examined.

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RESULTS

Patients and Eligibility

Thirty-five patients were enrolled. One patient was deemed ineligible because inadequate data were available. Patient characteristics are listed in Table 1. A majority of patients (58.8%) received two prior regimens. All patients had a performance status of 0 or 1.

Treatment and Response

Patients received a median of two cycles (range, 1-12) of protocol therapy. Of the 23 patients who received two or more cycles, 15 were escalated to 140 mg of dasatinib daily (70 mg bid) and two were reduced to 70 mg daily.

There were no responders. Also, only seven patients (20.6%; 90% confidence interval: 10.1%, 35.2%) were PFS-6 (Table 2). Therefore, the protocol criteria for continuing to the second stage of accrual were not met. Of the seven patients with PFS \geq 6 months, five had two prior treatment regimens while two had received one prior regimen, and all had platinum-resistant disease (<6 months platinum-free interval) Median PFS was 2.1 months (first and third quartiles: 1.8 and 4.9 months). Median overall survival (OS) was 17.7 months (first quartile: 10.8 months, third quartile has not been reached; Figure 1).

Toxicity

The most commonly observed grade 3 toxicities were gastrointestinal (mostly nausea and emesis), pulmonary (dyspnea and/or pleural effusion), and pain (Table 3). There were single cases of grade 3 or 4 anemia, malaise, insomnia, rash, and central nervous system hemorrhage.

163 Translational research

164 *Dasatinib related biomarkers*

165 Bead-based immunoassays were used to measure circulating levels of seven dasatinib
166 related biomarkers in plasma collected pre-cycles 1 (n=27), 2 (n=23), and 3 (n=15). Fifteen
167 patients submitted samples at all three time points. Median biomarker levels (ng/mL) are
168 presented (Supplemental Table S1). There was a significant increase in the levels of
169 sVEGFR2, sVGEFR3, and IGFBP2 between baseline and pre-cycle three in these patients.
170 There was no association between baseline levels of plasma biomarkers and outcome (PFS
171 and OS) nor between changes in biomarker levels and six-month PFS outcome (data not
172 shown).

173 *Circulating cfDNA*

174 Total cfDNA from plasma collected pre-cycles 1 (n=28), 2 (n=23), and 3 (n=15) was
175 quantified using a real-time PCR TaqMan Assay and primers directed to β -actin, β -globin, and
176 GAPDH. Median cfDNA totals (GE/mL) are presented (Supplemental Table S2). cfDNA
177 increased from baseline to pre-cycle 2 and decreased between pre-cycle 2 and pre-cycle 3;
178 these changes were not significant. There were no statistically significant associations between
179 baseline measures of cfDNA and outcome (PFS and OS) nor between changes in biomarker
180 levels and six-month PFS outcome (data not shown).

181 *Circulating Tumor and Endothelial Cells*

182 Whole blood was collected pre-cycles 1 (n=26), 2 (n=21), and 3 (n=12) for CTC and
183 CEC enumeration; CEC VEGFR expression was examined. Twenty-six patients were
184 evaluated for at least one time point; nine patients submitted samples at all three time points.
185 Median biomarker values are presented (Supplemental Table 3). Sixteen patients had ≥ 1 CTC

186 at one of the three timepoints, with nine patients having ≥ 1 at baseline. All patients had CECs
187 (range 14-800) at each timepoint tested. There were no statistically significant associations
188 between baseline CTCs or CECs and outcome (PFS and OS) nor between changes in
189 biomarker levels and six-month PFS outcome (data not shown).

190 CEC VEGFR expression was used to examine whether dasatinib had an effect on
191 endothelial cell activation and whether activation correlates with outcome. We anticipated the
192 percent of VEGFR+ CEC to decrease with a positive anti-angiogenic treatment effect. There
193 was no significant association between baseline CEC VEGFR expression and outcome (PFS
194 and OS) nor between changes in biomarker levels and six-month PFS outcome (data not
195 shown).

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DISCUSSION

198 Dasatinib was well tolerated but had minimal activity in patients with recurrent ovarian or
199 primary peritoneal carcinoma. With SRC reported to be overexpressed in approximately 90% of
200 ovarian cancers [3], no prescreening was performed to determine eligibility.

201 SRC occupies a strategic position in many cell signaling pathways affecting cell
202 proliferation, growth, and survival [4]. It has an important role in mediating the epithelial-to-
203 mesenchymal transition enhancing the metastatic potential of ovarian cancer cells [15,16].
204 Activated SRC is required for VEGF expression, which has already been shown to be important
205 in ovarian cancer biology and as an important therapeutic target. SRC expression was
206 associated with a drug resistant phenotype and SRC inhibition by transfecting SRC antisense
207 oligonucleotides (or by small molecule inhibitors) reversed drug resistance in preclinical models
208 [6]. Gene expression profiles of ovarian cancer tumors were able to identify with greater than
209 80% accuracy which tumors were likely to be resistant to primary platinum-based therapy
210 [26,27]. These profiles identified expression signatures consistent with activation of SRC which
211 in the future may be used to direct therapeutic strategies.

212 SRC protein expression usually correlated with the degree of SRC pathway
213 deregulation. The more deregulated the pathway, the higher the activated protein expression.
214 Platinum-resistant ovarian cancers have been shown to be more likely to have deregulation of
215 the SRC pathway [5,26]. Kaplan-Meier survival analysis showed that SRC pathway
216 deregulation is associated with a poor survival. Konecny and colleagues tested a panel of 34
217 ovarian cancer cell lines and reported that 71% were highly sensitive to dasatinib [28]. Teoh et
218 al, evaluated the *in vitro* activity of dasatinib alone and in combination with paclitaxel and
219 carboplatin in ovarian cancer cell lines [29]. Dasatinib demonstrated anti-proliferative activity
220 alone and synergistic activity with the cytotoxic in the cell lines with either SRC pathway

221 deregulation and/or high SRC protein expression. Dasatinib did not decrease SRC protein
222 expression, but completely abrogated the activation of SRC in all cell lines tested. These data
223 suggest dasatinib may help prime cells for apoptosis induced by cytotoxic chemotherapy.
224 Investigators at Duke University have conducted a phase I trial of carboplatin, paclitaxel, and
225 dasatinib [NCT00672295], but have yet to report these results.

226 It is not surprising that there was little single agent activity of dasatinib in this trial in light
227 of what has been learned about SRC since the conception and conduct of this trial. Preclinical
228 data support evaluating SRC inhibition in cancers with a predilection for developing metastases
229 to bone based on SRC's suppression of osteoclast function. A phase II trial of single agent
230 dasatinib investigated its activity in chemotherapy-naïve men with castrate resistant prostate
231 cancer (CRPC). A 43% stable disease rate (SDR) at 12 weeks and a 19 % SDR was
232 observed.(30) This level of activity was very similar to the results of current trial in patients with
233 recurrent ovarian cancer. A biochemical marker of drug activity was identified with a decline in
234 N-telopeptide, a marker of bone resorption predictive of adverse skeletal events, in men with
235 CRPC treated with dasatinib. Dasatinib has been similarly studied in patients with triple
236 negative breast cancer, Her 2 positive/hormone receptor positive breast cancer with response
237 rates of 4-5%. No or minimal activity also was observed in phase II trials of monotherapy
238 dasatinib in patients with head and neck cancer, glioma and small cell lung cancer (21,31).
239 None of these trials pre-selected patients based on any predictive biomarkers of dasatinib
240 activity. While SRC may be effective (under some circumstances) in suppressing tumor growth,
241 it is not able as monotherapy to cause regression of established tumors. There are
242 compensatory pathways that can bypass blockade of SRC. For example, SRC silencing results
243 in significant increase in FGR levels (another member of the SFK) [32]. Alternatively, activation
244 of JAK may re-establish downstream STAT signaling after inhibition of SFKs [33].

245 The low activity of dasatinib as a single agent should not preclude evaluation as part of
246 combination regimens, either concomitantly or in sequence prior to chemotherapy if it is borne
247 out that dasatinib can serve a priming function in ovarian cancers with a high degree of SRC
248 pathway deregulation. In this regard, Pathak and Godwin have recently completed *in vitro* high-
249 throughput screening (HTS) using a siRNA library targeting signaling molecules related to
250 receptor tyrosine kinases in combination with dasatinib. Such screening would identify second-
251 site molecules that can be targeted in combination with SRC inhibition to synergistically improve
252 dasatinib efficacy in patients and to identify a potential gene signature predictive of response to
253 dasatinib therapy (personal communication). The clinical significance of these genes identified
254 through the HTS as being capable of synergizing with SRC inhibition is now being evaluated in
255 blinded tumor biopsy samples from the patients that participated in this trial.

256 Our correlative studies did not show any significance between the biomarkers tested and
257 patient outcome, mainly due to no clinical response on this study. There was a significant
258 increase in the levels of sVEGFR2, sVEGFR3, and IGFBP2 between baseline and pre-cycle 3
259 in the 15 patients submitting samples at all three time points similar to the data reported by
260 Strauss and colleagues [34]. There were no statistically significant changes in the three
261 housekeeping genes from baseline in these patients. The mechanisms of the occurrence of
262 cfDNA in blood under normal and pathological conditions are not yet fully understood. cfDNA
263 might be influenced by apoptosis, necrosis, decreased DNAase activity in circulating cancer
264 cells, as well as clearance by liver/kidney, and modification status of cfDNA. In this study, 16
265 patients had ≥ 1 CTC at one of the three time points; 9 had ≥ 1 at baseline. Further investigation
266 of the relationship between cfDNA and circulating CTCs and the predictive value of changes in
267 CTC in patients with ovarian cancer is needed..

CONFLICT OF INTEREST

The co-authors have no conflicts of interest to declare.

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FIGURE LEGEND

Figure 1: Kaplan-Meier plots for overall and progression-free survival.