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ORIGINAL ARTICLE

Toll-like receptor-7 agonist administered subcutaneously in a prolonged dosing schedule in heavily pretreated recurrent breast, ovarian, and cervix cancers

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Abstract
Background The primary objective was to study the antitumor activity of prolonged subcutaneous dosing of systemic 852A, a Toll-like receptor-7 agonist (TLR-7), in recurrent breast, ovarian and cervix cancer. Secondary objectives included assessment of safety and immune system activation.
Methods Adults with recurrent breast, ovarian or cervix cancer failing multiple therapies received 0.6 mg/m^2 of 852A subcutaneously twice weekly for 12 weeks. Doses increased by 0.2 mg/m^2/week to a maximum of 1.2 mg/m^2. Serum was collected to assess immune activation.
Results Fifteen patients enrolled: 10 ovarian, 2 cervix and 3 breast. Three completed all 24 injections. There were two grade 2 (decreased ejection fractions), nine grade 3 (1 cardiovascular, 1 anorexia, 3 dehydration, 2 infections, 2 renal) and two grade 4 (hepatic and troponin elevation) unanticipated toxicities. Cardiac toxicities included three cardiomyopathies (2 asymptomatic) and one stress-related non-ST elevated myocardial infarction. Five patients discontinued therapy due to possibly associated side effects. One who had stable disease (SD) following 24 doses received 17 additional doses. A cervix patient with SD following 24 doses received chemotherapy after progressing 3 months later, and remains disease free at 18 months. Immune activation, as evidenced by increased IP-10 and IL-1ra, was observed.
Conclusions In this first human experience of a TLR-7 agonist delivered subcutaneously using a prolonged dosing schedule, 852A demonstrated sustained tolerability in some patients. Clinical benefit was modest, but immune activation was seen suggesting further study of antitumor applications is warranted. Because of cardiac toxicity; 852A should be used cautiously in heavily pretreated patients.

Keywords TLR-7 agonist · 852A · Ovarian cancer · Cervix cancer · Breast cancer

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Introduction

Although the immune system can control carcinogenesis [1], success with immunotherapy is limited in patients with established tumor, in part because of presumed inadequate activation of a coordinated response between the innate and adaptive immune systems. Components of infectious disease with antitumor activity have been identified as pathogen-associated molecular patterns, which activate the innate immune system through Toll-like receptors (TLRs). TLRs are preferentially expressed on cells of the innate

immune system, including dendritic cells (DC) [2]. Engagement of TLRs on DCs promotes cross-talk between the innate and the adaptive immune system, immune cell maturation, and migration of DCs into lymph nodes leading to activation, proliferation and survival of tumor antigen-specific $CD4^+$ and $CD8^+$ T cells. Because tumor cells usually do not express molecules that induce DC maturation, the application of TLR agonists may play an important role in activating T cells in cancer.

Imidazoquinolines (including imiquimod and resiquimod [R-848]) are low molecular weight synthetic compounds that have potent immune stimulating properties and have demonstrated antiviral and antitumor activity [3–5]. Molecule 852A (also known as S-32865, is *N*-[4-(4-amino-2-ethyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)butyl]methanesulfonamide) is a novel immune response modifier, related to the imidazoquinoline molecule imiquimod, that acts as a TLR-7 agonist. Imiquimod, brand name Aldara 5% cream™, has demonstrated antiviral and antitumor activity when topically applied to external genital and perianal warts, superficial basal cell carcinoma, actinic keratosis and metastases from malignant melanoma. The mechanism of immune activation by imidazoquinolines is via recognition by TLR-7 or TLR-8 and activation of a MyD88-dependent pathway. MyD88 is a Toll-interleukin 1 receptor domain-containing protein which recruits the IL-1 receptor-associated kinase and tumor necrosis factor receptor-associated factor 6 (TRAF6) to the TLR, which ultimately activates NF-κB transcription factors [6]. Imiquimod induces type I interferon (IFN) signaling [7] and has been shown to induce apoptosis in tissue culture [8].

The first human phase I trial of 852A was in patients with refractory solid organ tumors [9]. Drug was delivered intravenous (IV) and was limited to six doses, a duration too short to assess the response of the TLR-7 agonist. Recently, 852A's bioavailability, dose proportionality and tolerability were evaluated in a phase I study enrolling healthy volunteers [10]. Subjects received 852A by the IV, subcutaneous and oral (PO) routes. Absolute bioavailability for subcutaneous delivery determined over a 24-h period was approximately 80% and pharmacokinetic data for C_{max} and $AUC_{(0-t)}$ demonstrated dose proportionality for subcutaneous delivery. Therefore, we chose to test 852A subcutaneous delivery in refractory breast, ovarian and cervical malignancies to minimize patient burden and to allow for self-administration by patients who tolerated the drug for an extended period of time.

Materials and methods

Study criteria

We conducted a prospective, phase II trial at the University of Minnesota-Fairview Medical Center. Subjects were recruited August 2006–November 2007 July following Institutional Review Board approval and in accordance with the ethical standards in the 1964 Declaration of Helsinki. Female patients age >18 were eligible for enrollment if they had recurrent epithelial ovarian, fallopian tube or primary peritoneal disease or recurrent/persistent squamous or adenocarcinoma of the cervix failing two or more prior therapies. Breast cancer patients were enrolled if they had failed at least two previous chemotherapy or hormonal regimens for metastatic disease. Patients had to have had measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.0 with at least one target lesion >10-mm diameter, GOG performance score $\leq$2 or Karnofsky performance status $\geq$50 (breast). All patients had adequate organ function, a platelet count $\geq$50,000 μl^{-1}, a hemoglobin level $\geq$9 g/dl, and an absolute neutrophil count $\geq$1,000 μl^{-1}. Use of drugs known to induce QT interval prolongation and/or induce Torsades De Pointes was not allowed.

Treatment

Following informed consent, 852A (0.2% solution; 3 M pharmaceuticals, St. Paul MN) was administered subcutaneously twice weekly for 12 weeks (24 doses). The starting dose for all subjects was 0.6 mg/m^2. If no dose-limiting toxicity (described under toxicity assessments) or other symptoms of intolerance were observed after at least two consecutive injections, 852A was increased by 0.2 mg/m^2 increments to a maximum of 1.2 mg/m^2. BSA was calculated only once per course using the Dubois method. Patients with at least stable disease were allowed to receive additional 12-week treatment cycles.

Clinical assessments

Echo or multi gated acquisition (MUGA) scans were performed at baseline and at the end of therapy. Cardiac troponin levels were assessed at baseline and monthly. Clinical response was determined by RECIST Version 1.0 based on the tumor evaluation done at baseline and at completion of treatment at week 13 or earlier using computed tomography (CT), positron emission tomography and or magnetic resonance imaging scans.

Toxicity assessments

Toxicities were graded according to the National Cancer Institute (NCI) CTCAE Version 3. Toxicity assessments were performed within 1 month prior to dose 1, weekly until the patient reached their maximum tolerated dose and then every 2 weeks. Dose-limiting toxicities were any grade 3 or 4 non-hematologic toxicity with the following

exceptions: constitutional symptoms (transient chills, myalgia, arthralgia, headache, anorexia), nausea and vomiting controlled with antiemetics, grade 3 fever and grade 4 fatigue for <24 h. Hematological dose-limiting toxicities included ANC < 1,000 after three doses of G-CSF and thrombocytopenia of <30,000.

Immune monitoring

Immunologic monitoring included serum cytokine measurements from blood obtained pre- and 6-h post-dose 1, and for some patients mid-therapy (between doses 10 and 14), and at final dosage (dose 24). Serum cytokine/chemokine levels were determined by multiplex analysis on the Luminex platform (Austin, TX) using a 22-plex (human cytokine panel A) from R&D systems (Minneapolis, MN) and a 7-plex panel (for sCD40L, IL-12p70, IL-7, IL-12p40, IL-13, IL-15 and IP-10/CXCL10) from Millipore-Linco (Billerica, MA). Frequency and phenotype of lymphocyte populations were determined by flow cytometry analysis using methods previously described [9].

Statistics

Subjects had to receive at least 12 doses of 852A to be evaluable for clinical efficacy. Sample size was estimated prospectively for <5% type I error and 80% statistical power to detect a response rate of at least 30% in a two-stage design. The change in cytokine levels before and after treatment was compared using the Wilcoxon signed rank test. All analyses were performed using SAS version 9.1.

Results

Patient characteristics and clinical response

Fifteen patients were enrolled during the 15-month accrual period and 14 received treatment: 9 ovarian, 2 cervix and 3 breast (Table 1). The median number of prior chemotherapy regimens was 6.5 (range 2–16). The median age of subjects was 53 years (range 25–66), their mean weight was 76.7 kg (range 43–117), and 12 (86%) were Caucasian, and 2 (14%) black.

There were 7 evaluable patients who received at least 12 doses of 852A: 2 patients with cervix cancer and 5 with ovarian cancer. The median number of doses received was 12 (range 1–37). The reasons for discontinuation of treatment are shown in Table 2. 4 patients completed all 24 injections. 1, who had stable disease following 24 doses, recurred 3 months after completing therapy. She then received chemotherapy and remains disease free 18 months later. Another patient who had stable disease following 24 doses received 13 additional doses at 1.2 mg/m^2 until she developed anorexia, nausea and vomiting and discontinued therapy. 3 had at least 12 injections, but disease progression caused them to discontinue treatment before dose 24. Another ovarian cancer patient had stable disease following 10 injections but was taken off study due to fatigue.

Table 1 Demographics and tumor characteristics

Characteristic (N = 14)	
Age group, years (%)	
18–29	1 (7)
30–39	0 (0)
40–49	2 (14)
50–59	9 (64)
60–69	2 (14)
Age, median years (range)	53 (25–66)
Race (%)	
Caucasian	12 (85)
Black	2 (14)
Weight (kg), mean (range)	76.7 (43–117)
Primary site (%)	
Ovary	9 (64)
Cervix	2 (14)
Breast	3 (21)
GOG performance status (%)	
0	9 (64)
1	3 (21)
2	2 (14)
Prior treatment regimens, median (range)	6.5 (2–16)
Median doses received (range)	12 (0–37)
Received max dose of 1.2 mg/m^2 (%)	9 (64)
Total evaluable patients	
Cervix	2
Ovary	5
Breast	0

Toxicity

Unanticipated toxicities are summarized in Table 3. The 14 patients enrolled in this study had no grade 1 toxicities, 2 grade 2 toxicity (left ventricular dysfunction), 9 grade 3 (1 cardiovascular, 1 anorexia, 3 dehydration, 2 infections and 2 renal) and 2 grade 4 toxicities (hepatic and troponin elevation). Table 4 demonstrates the maximum grade targeted toxicity experienced for each patient in the trial. Pain was a commonly reported toxicity, with four patients experiencing grade 3 pain, however all events were attributed to disease. There were 12 patients who experienced malaise and 11 who experienced nausea during the trial. 5 patients discontinued therapy due to side effects possibly associated with 852A: 1 due to decreased ejection fraction, 1 due to a

Table 2 852A dose administration

Patient	Total doses received	Max dose received	Number of maximum doses received	Dose reduction	Treatment discontinued	Reason
1	24	1.2	14	No	No	
2	12	1.2	5	Yes	Yes	Disease progression
3	9	1.2	3	No	Yes	Disease progression
4	6	1	2	No	Yes	Nausea/vomiting
5	1	0.6	1	No	Yes	EF-15%
6	10	1.2	4	No	Yes	Fatigue
7	5	1	1	No	Yes	Disease progression
8	24	1.2	18	No	No	
9	12	1.2	6	No	Yes	Disease progression
10	37	1.2	31	No	During second course	Anorexia, nausea/vomiting
11	24	1.2	2	Yes	No	
12	2	0.6	2	No	Yes	Non-ST elevated MI
13	14	1.2	8	No	Yes	Disease progression
14	0					DOD before first dose

DOD dead of disease

Table 3 Summary of unanticipated drug-related adverse events

Category	CTCAE grade			
	1	2	3	4
Breast				
Cardiovascular	0	0	1	0
Total	0	0	1	0
Ovarian				
Troponin elevation	0	0	0	1
Left ventricular function	0	2	0	0
Anorexia	0	0	1	0
Dehydration	0	0	2	0
Elevated liver enzymes	0	0	0	1
Infection	0	0	1	0
Renal failure	0	0	1	0
Total	0	2	5	2
Cervix				
Dehydration	0	0	1	0
Infection	0	0	1	0
Renal	0	0	1	0
Total	0	0	3	0
Total grade 3 and 4 toxicity	0	2	9	2

CTCAE common terminology criteria for adverse events

Table 4 Maximum grade targeted toxicity

Category	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	6	5	0	0
Vomiting	1	5	0	0
Dyspnea	4	3	2	0
Fever	3	2	2	0
Chills	5	4	0	0
Myalgia	3	2	1	0
Sweats	5	2	0	0
Malaise	3	6	3	0
Edema	5	1	0	0
Cough	6	2	0	0
Pain	1	2	4	0
Total	42	34	12	0

non-ST elevated myocardial infarction (MI), 2 due to nausea and vomiting, and 1 other due to fatigue. Cardiac toxicity emerged as a particularly concerning unexpected toxicity, and the two DLT events and 2 asymptomatic patients identified during cardiac follow-up are described below in detail.

Patient 1 was a 45-year-old female diagnosed with an estrogen receptor-positive breast cancer, who developed grade 3 cardiomyopathy following her first 852A injection. At age 34, she underwent mastectomy followed by adjuvant chemotherapy, including doxorubicin. She had multiple recurrences and treatments which included additional anthracyclines. At study entry, the patient had metastatic lesions to the spine and liver. The patient had a MUGA scan prior to trial initiation that showed a left ventricular ejection fraction (LVEF) of 43%. Twelve hours following her first injection, she developed sudden substernal chest pain with significant shortness of breath, cough, vomiting and diarrhea during the night. Troponin was elevated at 0.56 without EKG changes, D-dimer was elevated at 3.1 and B-type natriuretic peptide was elevated at 3,020. Transthoracic echo demonstrated severely decreased LV function with an estimated ejection fraction of 15%. The patient was

managed with lasix, a beta-blocker, and an ACE inhibitor. After 14 days, her ejection fraction improved to 40%. By 2 months, her ejection fraction was 53%. This patient's 6-h post-dosing IP-10, IL-1ra and MCP-1 cytokine levels rose significantly from 235.1 to 5,045.2 pg/ml, 624 to 20,151.3 pg/ml and 257 to 1,696 pg/ml, respectively. The increases in IP-10 and IL-1ra were the second largest observed among this cohort of patients.

Patient 2 was a 47-year-old woman with platinum-resistant stage IIIc ovarian carcinoma who received adjuvant paclitaxel and carboplatin, consolidation with docetaxel followed by liposomal doxorubicin. Her pre-study MUGA was 72%. Two days prior to her first injection, the patient had diarrhea and had received IV fluids for dehydration. Two days following her first injection, she was admitted to the hospital with dehydration, acute renal failure and weakness. The troponin on admission was 4.33 μg/L. During her hospitalization, her creatinine rose to 3.44 mg/dl with an ALT of 140 μ/l and AST of 736 μ/l, uric acid of 12 mg/dl, a phosphorus of as 6.3 mg/dl and a decrease in calcium to 6.9 mg/dl. An ECG revealed sinus tachycardia and the patient was admitted to cardiology with diagnosis of a non-ST elevation MI and possible tumor lysis syndrome. A MUGA was repeated, showing a LVEF of 80%. The patient was discharged on hospital day 10 with resolution of her laboratory abnormalities. This patient's 6-h post-dosing IP-10, IL-1ra and MCP-1cytokine levels rose significantly from 1,017.5 to 5,119.6 pg/ml, 3,145.2 to >42,000 pg/ml and 189.9 to 6,185.8 pg/ml, respectively.

Patient 3 was a 54-year-old diagnosed with stage IIIc serous ovarian cancer who received adjuvant paclitaxel and carboplatin followed by altretamine consolidation. Following recurrence, she went on to receive topotecan followed by liposomal doxorubicin, gemcitabine, cisplatin and paclitaxel. Prior to beginning the 852A study her LVEF was 51%. Following completion of 24 doses, it had decreased to 42.6%. She was managed with an ACE inhibitor and a beta-blocker. After 3 months, her LVEF was 53%. She died of progressive disease 4 months later.

Patient 4 was a 52-year-old patient diagnosed with stage IIIC serous ovarian cancer who, after receiving adjuvant paclitaxel and carboplatin, recurred within 3 months and started liposomal doxorubicin. She went on to receive gemcitabine, topotecan, 5-FU plus oxaliplatin plus leucovorin, cyclophosphamide, etoposide and doxorubicin. She received her last dose of doxorubicin 3 weeks prior to starting 852A. Her pre-study LVEF was 68%. Owing to the previously observed cardiotoxicity, a MUGA was obtained 2 months after initiating treatment, following her 16th injection. Her LVEF decreased to 57% and subsequently to 48% following her 24th injection. The patient remained without evidence of heart failure and had stable disease. She desired a second course of TLR-7 agonist. After 1 month of completion of her first 24 doses, a repeat MUGA showed a LVEF of 60% and the patient elected to start a second course of therapy. She received 17 injections and discontinued the study due to disease progression during which she did not experience any additional cardiac issues.

Immunologic effects of 852A

We evaluated a panel of cytokines thought to be involved in the DC NK pathway. There was a statistically significant increase in IL-1ra and IP-10, with a 2.6- and 4.9-fold change, respectively observed from baseline to 6 h following the first dose of 852A (Table 5). The increase in IL-1ra and IP-10 levels was maintained after repeated doses with no evidence of diminished responsiveness (Fig. 1). Based on our results, increases in cytokine measurements are short lived and there was no evidence of cytokine accumulation, but responsiveness was maintained mid-therapy at levels similar to initial dosing. Lymphocyte subsets were

Table 5 Cytokine median values and ranges pre-treatment and 6-h post-treatment dose 1

Cytokine	*N*	Median (range)	Median fold change (range)	*p* value
IL-1ra				
Pre-treatment	13	903.30 (420.37, 4,591.80)		
6 h after treatment	12	2,981.15 (505.29, 42,000)		
Difference	12	1,902.16 (−60.67, 38,854.80)	2.6 (0.95, 32.3)	0.0024*
IP-10				
Pre-treatment	13	210.60 (71.44, 1,017.52)		
6 h after treatment	12	1,129.79 (176.12, 5,164.90)		
Difference	12	856.84 (34.53, 4,810.08)	4.9 (1.1, 21.5)	0.0005*

IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, ENA-78/CXCL5, FGF-basic, G-CSF, GM-CSF, IFN-γ,MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, RANTE S/CCL5, TNF-α, TPo, VEGF, sCD40L, IL-12p70, IL-7, IL-12p40, IL-13, IL-15 and IP-10/CXCL10 were analyzed

The table represents the cytokines that were significantly different between pre and 6 h post 852 dose 1

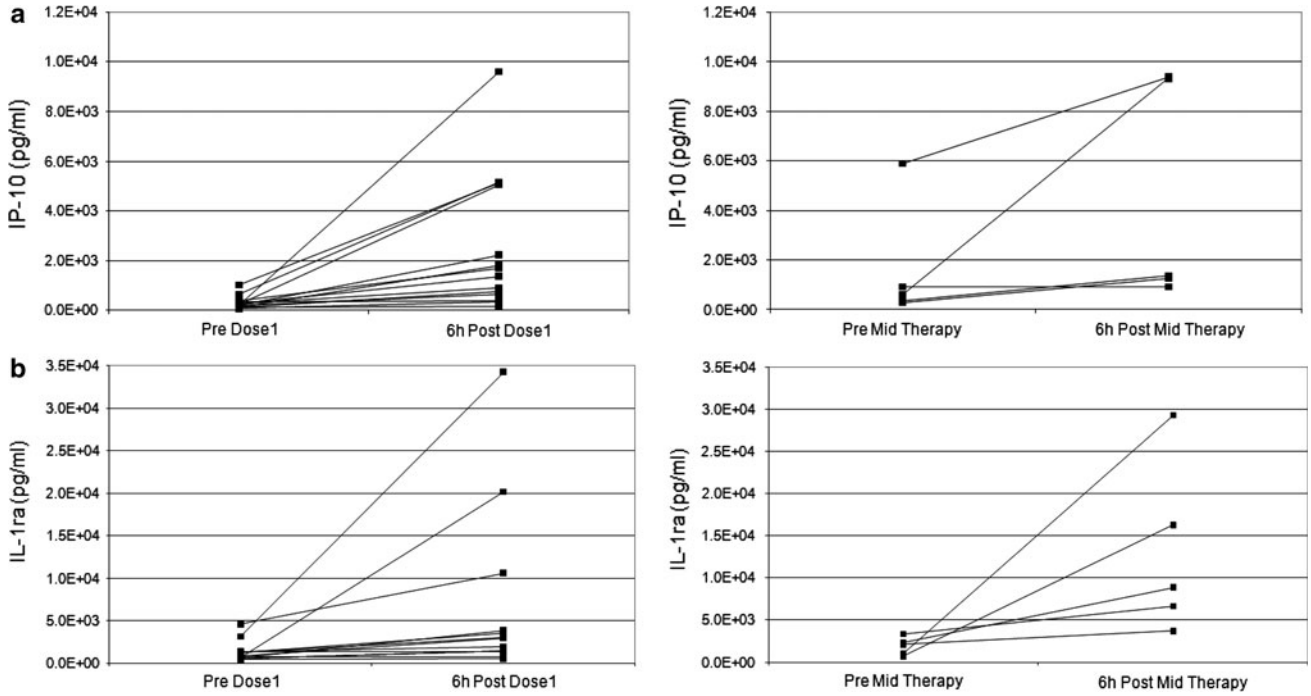

Fig. 1 IP-10 levels (pg/ml) (**a**) and IL-1ra levels (pg/ml) (**b**) prior to 852A injection and 6-h post-injection at dose 1 and at mid-therapy dose

evaluated at baseline and 6 h after the first dose of 852A to determine if NK cell activation occurred. No lymphocyte subsets changed more than a median of twofold. For a subset of patients (4/13) CD69, a marker of NK cell activation was significantly increased 6 h after the first dose of 852A as compared to baseline levels (data not shown). There was no correlation between NK cell activation and IL-10 and IL-1ra levels.

Discussion

852A can be delivered subcutaneously using a prolonged dosing schedule up to 1.2 mg/m^2 twice weekly. Evidence of clinical activity and immune activation was present in our advanced disease cohort. Although all toxicities attributed to the study drug were reversible, the cardiac events were unexpected and warrant cautious use of 852A in heavily pretreated patients. The majority of patients experienced malaise, a side effect which has been associated with other TLR agonists.

852A directly stimulates cells in the innate immune system, via the TLR-7 pathway, resulting in DC maturation, induction of multiple cytokines including IFN-α, and enhanced antigen presentation, [11] making 852A desirable for therapeutic application. Dudek et al. [9] established the maximum tolerated IV dose of 1.2 mg/m^2 of 852A three times weekly in their phase I study. Similar to our trial using subcutaneous dosing, several studies observed increases in IP-10 and IL-1ra biomarkers following dosing of at least 0.6 mg/m^2 [9, 12]. Although levels of IP-10 and IL-1ra rose 6 h following the first injection and after repeat injections, levels did not continually rise. IP-10 has been shown to promote induction of T helper 1 cytokines necessary for antitumor response and represents a downstream marker for the presence of IFN-α, a biomarker associated with TLR-7 agonists [13–15]. Although it was not statistically elevated following injection, MCP-1 has been shown to augment NK cell cytolysis in vitro and induce potent NK cell migration [16]. Changes in the activation markers (IP-10 and IL-1ra) associated with TLR-7 agonists support the proposed pharmacologic activity of 852A as a selective TLR-7 agonist as has been observed by others [9]. Although we did not see any correlation between increased IP-10 and IL-1ra levels and NK cell activation, it is possible that decreased NK cells are in sampled blood, as a result of the activation process and egress of lymphocytes into tissue. We acknowledge that monitoring peripheral blood may not accurately detect NK cell activation and that tissue sampling may better represent the state of NK cells following exposure to an immune modulating drug such as 852A. Trafficking studies will be an important adjunct to future studies.

The tumors we focused on in this study had potential for immune-based responses. Breast cancers are immunogenic tumors and most patients demonstrate cellular and humoral immune responses [17]. A recently published report of an off-label use of Imiquimod (Aldara) to treat cutaneous

breast cancer metastases described two women who had complete resolution of their cutaneous disease after topical application of the imidazoquinoline cream [18]. We chose to study 852A in an ovarian cancer population given a history of strategies testing immune responses, including the use of other TLR agonists and tumor-derived exosomes carrying tumor-associated antigens, in advanced ovarian cancer patients [19]. The cervix cancer population is an obvious target for 852A as more than 90% of cervix cancer cases are known to be the result of infection with high-risk HPV types, such as 16 and 18. Imiquimod (Aldara), another TLR-7 agonist, has shown efficacy in the treatment of external genital warts often caused by HPV 6 and 11 as well as recurrent high-grade intraepithelial neoplasia of vulva, vagina and cervix [20].

The cardiac adverse events observed with 852A administration have not previously been reported. A known potential side effect of 852A is prolongation of action potential in hERG potassium-ion channels. This prolongation has been observed in vitro at 3,000 ng/ml of 852A with QTc increases in dogs at 2.5 mg/kg [21]. No adverse trends in electrocardiogram QTc measurements were reported by Dudek or Dummer [9, 21] nor in our patients. The decreases in LVEF and non-ST elevation MI seen in our cohort have not previously been reported with 852A. The patient with the most extreme cardiomyopathy had received doxorubicin 10 years prior to the study and, among multiple other regimens, had received two additional courses of liposomal doxorubicin 1 year and again 1 month prior to study entry. 6 h following her first 852A injection, this patient had large increases in her serum cytokine levels. Her IP-10 level was 21 times, IL-1ra 32 times and MCP-1 6.6 times that of baseline. The decrease in ejection fraction, which resolved within 3 months, may be a result of acute immune activation and cytokine storm after a single 852A dose. Similarly, the ovarian cancer patient who experienced the non-ST elevation MI in the setting of tumor lysis had significant increases in her cytokine levels after 852A dosing. Further investigation of the immune responses seen in patients who developed cardiotoxic events is warranted. The significant increases in cytokines observed in the 2 symptomatic patients were not seen in the patients who experienced transient asymptomatic decreases in their ejection fraction.

Although a true efficacy rate could not be determined, a key clinical observation was of disease stabilization in two heavily pretreated patients. One ovarian cancer patient with platinum refractory disease had received eight prior therapies before starting 852A. Following 24 doses of the drug, she had stable disease and went on to receive 13 additional doses before disease progression. The other patient with stable disease had stage IIIB squamous cell cervix cancer and had received radiation and two prior chemotherapy regimens. She was found to have stable disease following 24 doses of drug, but progressed 3 months following her last injection. At the time of her progression, she was placed on chemotherapy and is without evidence of disease 18 months later. This is an unusual case as response rates in cervical cancer patients with recurrent disease or pelvic metastases are poor, and 1-year survival rates are between 15 and 20% [22]. This patient is currently 27 months from trial completion which begs the question of whether TLR-7 agonists, as initiators of innate and adaptive immune responses, could have "primed" the tumor cells for the next therapy, resulting in the unlikely effective antitumor response observed. The stable disease observed following use of 852A leads us to believe that combination therapy would be of potential interest for future phase II studies.

In summary, we report the first subcutaneous administration of 852A, a selective small-molecule TLR-7 agonist, in patients with recurrent ovarian, cervix, and breast cancers. Grade 3 and 4 toxicities were short lived and manageable. The frequency and severity of cardiac toxicity, while apparently reversible, exceeded expectations and may limit the development of this agent. However, we did find evidence of immune activation and disease stabilization in 2 patients suggesting that 852A may have a role for future systemic antitumor studies. Immunotherapy alone is unlikely to have a major impact on a disease associated with bulky tumor, as is frequently the situation in recurrent solid tumors. The limited efficacy we observed with 852A monotherapy in our heavily pre-treated, advanced disease population is not surprising. Optimizing this therapy may require integration into frontline treatment or as a vaccine adjuvant to maximize T cell and NK activation. Additional preclinical and clinical studies would be necessary to clarify what role TLR-7 agonists could play as antitumor treatments.

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Conflict of interest The authors declare that they have no conflict of interest

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