

# A Phase 1B/2 Study of Aldoxorubicin in Patients With Soft Tissue Sarcoma

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**BACKGROUND:** Aldoxorubicin, a prodrug of doxorubicin, covalently binds to serum albumin, allowing for the administration of much higher doses of doxorubicin in a previous clinical study. The current phase 1B/2 study evaluated the safety of aldoxorubicin, including preliminary efficacy and safety of its maximum tolerated dose (MTD). **METHODS:** Patients aged 18 to 70 years with recurrent/refractory malignant solid tumors received aldoxorubicin at a dose of 230 mg/m<sup>2</sup>, 350 mg/m<sup>2</sup>, or 450 mg/m<sup>2</sup> (170 mg/m<sup>2</sup>, 260 mg/m<sup>2</sup>, or 335 mg/m<sup>2</sup> doxorubicin equivalents, respectively) by intravenous infusion once every 21 days for up to 8 consecutive cycles. **RESULTS:** A total of 25 patients were enrolled, including 17 patients (68%) with advanced soft tissue sarcoma (STS). The MTD of aldoxorubicin was 350 mg/m<sup>2</sup>; dose-limiting toxicities included grade 4 neutropenia and grade 3 febrile neutropenia (NCI CTCAE v4.0). Drug-related adverse events included myelosuppression, nausea, fatigue, alopecia, stomatitis, vomiting, and oropharyngeal pain. No clinically significant cardiac toxicities were reported. Seven patients (28%) had elevated serum troponin levels while taking part in the study, but these elevations were not clinically significant or associated with cardiac findings. A partial response was achieved in 20% of patients, and stable disease was reported in 40% of patients. The median progression-free survival was 4.80 months, and the median overall survival was 11.25 months. Among patients with STS who were treated at the MTD (13 patients), a partial response was achieved in 38% and stable disease in 46%; the median progression-free survival was 11.25 months and the median overall survival was 21.71 months. **CONCLUSIONS:** Aldoxorubicin at a dose of 350 mg/m<sup>2</sup> administered once every 21 days for up to 8 cycles was found to be acceptably safe and demonstrated preliminary efficacy in patients with advanced solid tumors, including STS. Further investigation of aldoxorubicin is ongoing. *Cancer* 2014;000:000-000. © 2014 The Authors. *Cancer* published by Wiley Periodicals, Inc. on behalf of *American Cancer Society*. This is an open access article under the terms of the Creative Commons Attribution NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

**KEYWORDS:** doxorubicin, maximum tolerated dose, prodrugs, serum albumin, soft tissue sarcoma.

## INTRODUCTION

An estimated 11,410 individuals in the United States were diagnosed with soft tissue sarcoma in 2013.<sup>1</sup> In 39% of newly diagnosed cases, the cancer is found to have spread regionally (24%) or distally (15%), with corresponding 5-year relative survival rates of 61% and 18%, respectively.<sup>2</sup>

Doxorubicin-based chemotherapy is a standard of care for patients with advanced, unresectable, or metastatic soft tissue sarcoma.<sup>3</sup> Although effective in the treatment of a variety of cancers, including advanced soft tissue sarcoma,<sup>4-8</sup> the advantages of doxorubicin monotherapy and dose-intensive, doxorubicin-based combination regimens are mitigated by dose-related toxicities, particularly cardiomyopathy and congestive heart failure.<sup>9,10</sup> Even when the total cumulative dose of doxorubicin is limited, late-onset cardiotoxicity has been observed.<sup>11,12</sup> The administration of dexrazoxane may provide cardioprotection in patients receiving cumulative doses of doxorubicin-based therapy of  $\geq 300$  mg/m<sup>2</sup> but requires careful observation.<sup>13</sup> Consequently, high-dose or long-term treatment with doxorubicin is not generally feasible.

Aldoxorubicin is a prodrug of doxorubicin that is derivatized at its C-13 keto-position with a thiol-binding spacer molecule (6-maleimidocaproic acid hydrazide) (Fig. 1A).<sup>14,15</sup> When aldoxorubicin enters the bloodstream, this spacer

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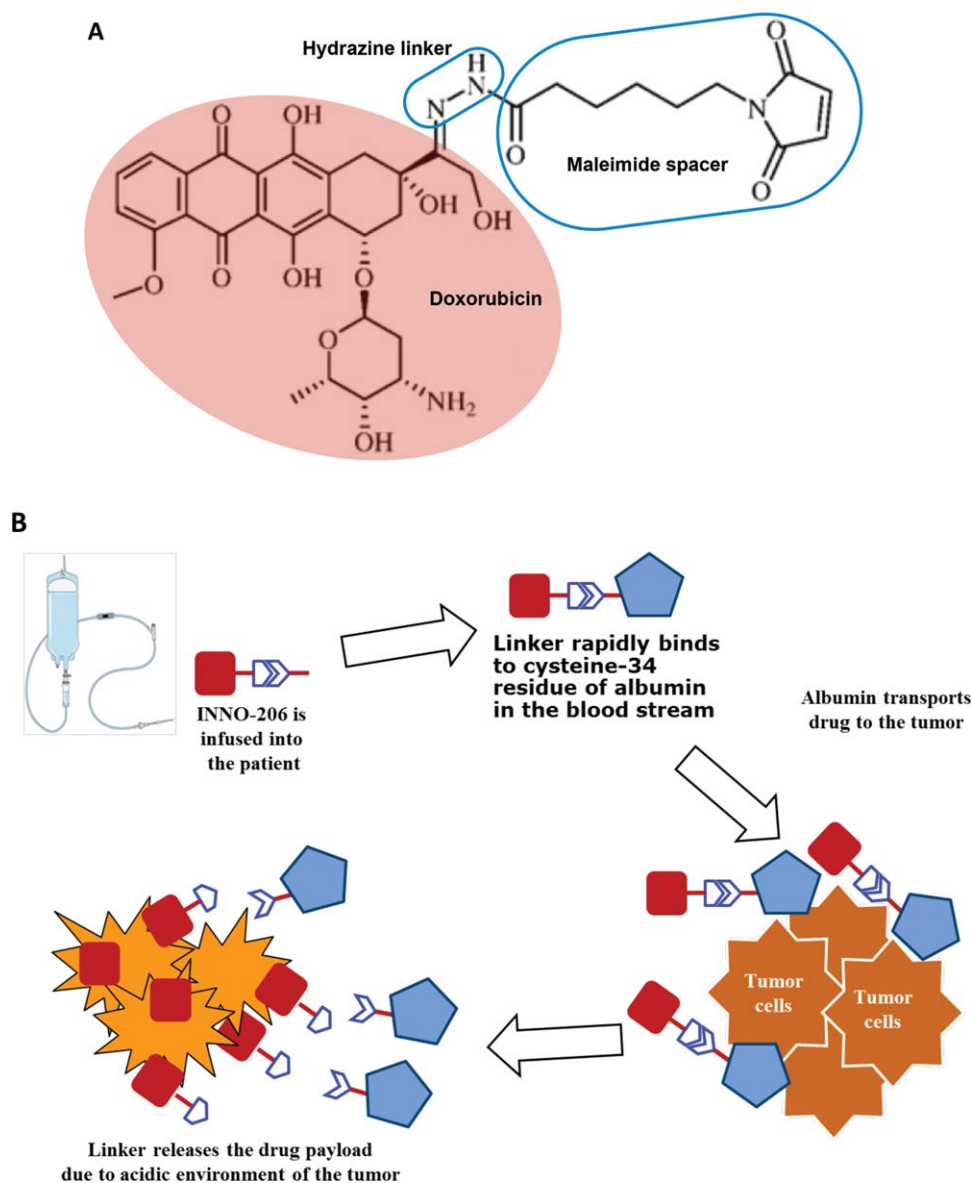
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**Figure 1.** Proposed mechanism of action of aldoxorubicin is shown. (A) The chemical structure of aldoxorubicin (INNO-206; EMCH-doxorubicin) is shown. Adapted from Kratz F. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. *J Control Release.* 2008;132:171-183.<sup>15</sup> (B) The following are the steps involved in its mechanism of action. Step 1: aldoxorubicin (red square with linker/spacer) is infused into the patient. Step 2: the maleimide spacer rapidly reacts with the thiol group of cysteine-34 of endogenous albumin (blue pentagon). Step 3: albumin transports the drug complex to the tumor. Step 4: the acidic environment of the tumor leads to cleavage of the hydrazine linker, thereby releasing the doxorubicin drug payload at the site of the tumor.

molecule rapidly and covalently binds to only the most reactive thiol group in human plasma, the cysteine-34 amino acid of endogenous albumin. The albumin-bound doxorubicin is then carried to the tumor, and doxorubicin is released in the acidic environment of the tumor via cleavage of an acid-sensitive hydrazine bond between the drug and the carrier (Fig. 1B).<sup>14,15</sup>

In toxicological animal studies, the median lethal dose of aldoxorubicin in mice and rats was 2 to 5 times

that of doxorubicin, and the maximum tolerated dose (MTD) of aldoxorubicin in dogs was 3 times that of doxorubicin.<sup>16</sup> Furthermore, the exposure of rats to aldoxorubicin was associated with less clinical and histopathological evidence of cardiotoxicity compared with equimolar dosing of doxorubicin,<sup>17</sup> which might be explained by other experiments demonstrating lower accumulation of aldoxorubicin in organs, including the heart, compared with native doxorubicin.<sup>15</sup>

In a phase 1 study of aldoxorubicin in patients with advanced solid tumors, dose-limiting toxicities (DLTs) of mucositis and neutropenia were observed at the MTD (340 mg/m<sup>2</sup> doxorubicin equivalents) and at the recommended dose of 260 mg/m<sup>2</sup> doxorubicin equivalents. It is important to note that no clinical signs of cardiotoxicity were observed, even among patients in the higher dose groups. Partial remission was observed in 3 patients, including 1 with a soft tissue sarcoma (liposarcoma) and 2 with solid organ tumors (small cell lung cancer and metastatic breast cancer, respectively).<sup>18</sup>

Since then, a new formulation of aldoxorubicin has been developed. The current study evaluated the safety of the new formulation of aldoxorubicin in an abbreviated phase 1B/2 dose escalation study, as well as the preliminary efficacy and safety of aldoxorubicin at its MTD in patients with advanced soft tissue sarcoma who had developed disease progression while receiving prior chemotherapies.

## MATERIALS AND METHODS

### *Study Design*

The current study was a phase 1B, open-label study to evaluate the safety of the new formulation of aldoxorubicin administered at doses of 230 mg/m<sup>2</sup>, 350 mg/m<sup>2</sup>, and 450 mg/m<sup>2</sup> (170 mg/m<sup>2</sup>, 260 mg/m<sup>2</sup>, and 335 mg/m<sup>2</sup> doxorubicin equivalents, respectively) by intravenous infusion on day 1 of every 21-day cycle for a maximum of 6 consecutive cycles (later amended to 8 cycles). The new formulation of aldoxorubicin no longer uses a bulking agent or a viscosity-reducing agent. It also has fewer buffering agents and no longer needs pH adjustment or cold storage at 5°C before infusion. The diluent was simplified to a 50:50 mix of ethanol:water, the use of which allows a 2-hour window for reconstitution and drug delivery at room temperature. The primary endpoint was to evaluate treatment-related toxicities and determine the MTD, and the secondary endpoint was to determine the objective response rate (ORR) using version 1.1 of the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) after treatment with aldoxorubicin. Escalation to the next dose level occurred if fewer than 2 of 5 or fewer than 4 of 8 patients experienced a DLT (grade 3 or 4 nonhematologic toxicity, excluding alopecia and nausea/vomiting; platelet count <25,000 cells/μL or neutrophil count <500 cells/μL lasting >7 days; and/or associated with a fever >38.5°C) after completing cycle 1 and before starting cycle 2 of study treatment. If at least 2 of 5 or at least 4 of 8 patients experienced a DLT, then 3 additional patients were enrolled at that dose level. If 2 of the 3 additional patients experienced a DLT, the previous dose level would

be defined as the MTD. After establishment of the MTD, the MTD cohort was expanded to a total of 18 patients to provide additional safety information. This study, clinical trial identifier NCT01337505,<sup>19</sup> was conducted in accordance with current US Food and Drug Administration regulations, International Conference on Harmonisation Good Clinical Practice guidelines, the principles of the Declaration of Helsinki, and other applicable regulations and guidelines.

### *Patients*

Patients were aged 18 to 70 years with malignant solid tumors that had recurred or were refractory to standard therapy; had stable brain metastases, if present on computed tomography or magnetic resonance imaging scans during screening; had an Eastern Cooperative Oncology Group performance status of 0 to 2; had a life expectancy >12 weeks; were not pregnant or lactating (women patients only); had received no palliative surgery, chemotherapy, immunotherapy, and/or radiotherapy within 4 weeks of the screening visit, and had no exposure to any investigational agent within 30 days of the screening visit; had adequate liver and bone marrow function; had no clinically evident congestive heart failure worse than New York Heart Association functional class II; had no serious, clinically significant cardiac arrhythmias, no history or signs of active coronary artery disease, and no serious myocardial dysfunction or left ventricular ejection fraction <45% of predicted; had no active, clinically significant, serious infection, including human immunodeficiency virus, that required treatment; and had undergone no major surgery within 3 weeks of the first dose of the study drug. All patients provided written informed consent.

### *Safety Assessments*

At baseline and on day 1 of each cycle, safety monitoring (including adverse events [AEs]), physical examination, serum chemistry (including troponin I), complete blood count, urinalysis, and electrocardiography were performed. Additional cardiac monitoring by echocardiography or multigated acquisition scan was performed at baseline, on days 28 and 85, and during the follow-up period. AEs were graded using a descriptive scale within the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0.

### *Efficacy Assessments*

At baseline, on days 28 and 85, and during the follow-up period, tumor measurements were made. Objective response was evaluated using RECIST 1.1 criteria. Measurable reductions in tumor dimensions from baseline values

**TABLE 1.** Patient Demographics and Baseline Characteristics

| Characteristic                                    | Initial Aldoxorubicin Dose Cohort |                                  |                       |                                  |
|---|-----------------------------------|----------------------------------|-----------------------|----------------------------------|
|   | 230 mg/m <sup>2</sup>             | 350 mg/m <sup>2</sup>            | 450 mg/m <sup>2</sup> | All Patients                     |
| No. of patients                                   | 5                                 | 18 <sup>a</sup>                  | 2 <sup>a</sup>        | 25                               |
| Median age (range), y                             | 55.1 (28-68)                      | 56.2 (25-70)                     | 42.5 (40-45)          | 55.1 (25-70)                     |
| Male/female, no. (%)                              | 2/3 (40/60)                       | 9/9 (50/50)                      | 0/2 (0/100)           | 11/14 (44/56)                    |
| Race, no. (%)                                     |                                   |                                  |                       |                                  |
| White   | 5 (100)                           | 16 (89)                          | 2 (100)               | 23 (92)                          |
| Black or African American                         | —                                 | 1 (6)                            | —                     | 1 (4)                            |
| Asian   | —                                 | 1 (6)                            | —                     | 1 (4)                            |
| ECOG performance status, no. (%)                  |                                   |                                  |                       |                                  |
| 0   | —                                 | 2 (11)                           | —                     | 2 (8)                            |
| 1   | 5 (100)                           | 16 (89)                          | 2 (100)               | 23 (92)                          |
| 2   | —                                 | —                                | —                     | —                                |
| Primary tumor site, no. (%)                       |                                   |                                  |                       |                                  |
| Bone metastases                                   | —                                 | 1 (6)                            | —                     | 1 (4)                            |
| Cervix  | —                                 | 1 (6)                            | —                     | 1 (4)                            |
| Head and neck                                     | —                                 | —                                | 1 (50)                | 1 (4)                            |
| Kidney  | —                                 | 1 (6)                            | —                     | 1 (4)                            |
| Liver   | 1 (20)                            | —                                | —                     | 1 (4)                            |
| Ovary   | 1 (20)                            | —                                | —                     | 1 (4)                            |
| Prostate  | —                                 | 1 (6)                            | —                     | 1 (4)                            |
| Soft tissue                                       | 1 (20)                            | 4 (22)                           | —                     | 5 (20)                           |
| Other   | 2 (40)                            | 10 (56)                          | 1 (50)                | 13 (52)                          |
| TNM stage, no. (%)                                |                                   |                                  |                       |                                  |
| III   | 4 (80)                            | 1 (6)                            | —                     | 5 (20)                           |
| IV  | 1 (20)                            | 17 (94)                          | 2 (100)               | 20 (80)                          |
| Median no. of prior chemotherapy regimens (range) | n = 5<br>3.0 (1-4)                | n = 16 <sup>b</sup><br>3.0 (1-7) | n = 2<br>1.5 (1-2)    | n = 23 <sup>b</sup><br>3.0 (1-7) |

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

<sup>a</sup>One patient initially received a dose of 450 mg/m<sup>2</sup> in cycle 1 and subsequently received 350 mg/m<sup>2</sup> in cycles 2 through 8.

<sup>b</sup>Two patients in the group treated at a dose of 350 mg/m<sup>2</sup> had received no prior medication or chemotherapy.

were confirmed within 8 weeks after first documentation of response. Complete response (CR) was defined as the disappearance of all target lesions and a partial response (PR) was defined as a  $\geq 30\%$  decrease from baseline in the sum of the longest diameter of all target lesions and no new lesions. After a PR or CR was confirmed, tumors were measured every 3 months until the end of the study.

### Statistical Analysis

Patients who had received at least 1 dose of the study drug were included in the primary safety analyses. Patients who had received at least 1 dose of study drug and had undergone at least 1 postdose tumor assessment were evaluable for tumor response. ORR, as well as the rates of CR, PR, stable disease (SD), and progressive disease, were estimated by the percentage of patients meeting the criteria for each level of response. Kaplan-Meier analyses were used to estimate progression-free survival (PFS) and overall survival (OS).

## RESULTS

### Patients

A total of 25 patients were enrolled in the current study: 5 patients in the 230 mg/m<sup>2</sup> dose group (cohort 1); 6

patients in the 350 mg/m<sup>2</sup> dose group, which was later expanded to a total of 18 patients (cohort 2); and 2 patients in the 450 mg/m<sup>2</sup> dose group (cohort 3). One of the 2 patients initially enrolled in cohort 3 completed 1 cycle at the 450 mg/m<sup>2</sup> dose level and subsequently completed 7 cycles at the 350 mg/m<sup>2</sup> dose level. The other patient in this cohort was withdrawn from the study after the first cycle because of liver failure secondary to disease.

Demographics and baseline characteristics of the study patients are summarized in Table 1. Cohorts differed with regard to sex and racial demographic characteristics, but other patient and disease characteristics were similar across cohorts. Seventeen patients (68%) had a diagnosis of soft tissue sarcoma, most commonly of the leiomyosarcoma histologic subtype (12 patients). Twenty-three patients had received a median of 3 prior chemotherapy regimens (range, 1-7 regimens); of these, 12 patients (52%) had received prior doxorubicin, pegylated liposomal doxorubicin, and/or epirubicin.

### Safety Evaluations

To further investigate the safety and tolerability of aldorubicin at the MTD, cohort 2 was expanded and patients in this cohort were allowed to receive an additional 2 cycles of therapy, for a total of 8 cycles.

**TABLE 2.** Summary of Study Drug-Related TEAEs

|   | Initial Aldoxorubicin Dose Cohort |   |  | All patients<br>(N = 25) |
|---|-----------------------------------|---|--|--------------------------|
|   | 230 mg/m <sup>2</sup><br>(n = 5)  | 350 mg/m <sup>2</sup><br>(n = 18 <sup>a</sup> ) | 450 mg/m <sup>2</sup><br>(n = 2 <sup>a</sup> ) |                          |
| No. of study drug-related TEAEs   | 35                                | 302   | 60   | 397                      |
| Patients with study drug-related TEAEs, no. (%)                                   | 4 (80)                            | 18 (100)  | 2 (100)  | 24 (96)                  |
| Patients with study drug-related TESAEs, no. (%)                                  | —                                 | 3 (17) <sup>b</sup>                             | 2 (100)  | 5 (20) <sup>a</sup>      |
| Patients who discontinued study drug because of study drug-related TEAEs, no. (%) | 1 (20)                            | 2 (11)  | 1 (50)   | 4 (16)                   |
| Deaths, no. (%)   | —                                 | 1 (6)   | —  | 1 (4)                    |

Abbreviations: TEAE, treatment-emergent adverse event; TESA, treatment-emergent serious adverse event.

<sup>a</sup>One patient initially received a dose of 450 mg/m<sup>2</sup> in cycle 1 and subsequently received a dose of 350 mg/m<sup>2</sup> in cycles 2 through 8.

<sup>b</sup>Includes 1 death.

The median number of cycles completed was 4 cycles (range, 1-8 cycles) for the entire safety population. For each cohort, the median number of cycles completed was 2 cycles (range, 2-5 cycles) in cohort 1, 6 cycles (range, 1-8 cycles) in cohort 2, and 1 cycle in cohort 3. Patients with soft tissue sarcoma in cohort 2 (13 patients) completed a median of 8 cycles (range, 2-8 cycles).

No DLT was observed in cohort 1. Of the first 6 patients enrolled in cohort 2, 1 patient experienced DLTs (dehydration, sepsis, and neutropenia). The first 2 patients in cohort 3 experienced DLTs (grade 4 neutropenia and grade 3 febrile neutropenia), and the principal investigator deemed it unsafe to enter more patients at this dose level. The MTD was determined to be 350 mg/m<sup>2</sup> (or 260 mg/m<sup>2</sup> doxorubicin equivalents).

A summary of study drug-related treatment-emergent AEs (TEAEs) is shown in Table 2, and the incidences of study drug-related TEAEs are shown in Table 3. Myelosuppression (mostly grade 3 or 4 neutropenia, mostly grade 1-3 thrombocytopenia, and mostly grade 1 or 2 anemia) was the most common study drug-related AE observed during treatment. Fatigue, alopecia, gastrointestinal-related events (nausea, vomiting, decreased appetite, constipation, and gastroesophageal reflux disease), and stomatitis-related events (mouth ulcerations, oral pain, and oropharyngeal pain) were common study drug-related, nonhematologic AEs. These nonhematologic AEs were mostly grade 1 or 2 in severity.

A total of 6 patients (1 patient in cohort 1, 4 patients in cohort 2, and 1 patient in cohort 3) discontinued the study drug because of a TEAE. For 2 of these patients (both of whom were in cohort 2), the TEAEs leading to treatment discontinuation (grade 2 hypophosphatemia and grade 2 pyelonephritis and grade 3 fatigue, respectively) were not considered by the investigator to be causally related to the study drug. For the other 4 patients (1 patient in cohort 1, 2 patients in cohort 2, and 1 patient in cohort 3), the TEAEs

leading to discontinuation (grade 3 fatigue, grade 4 thrombocytopenia, grade 5 septic shock, and grade 3 anemia and grade 3 neutropenia, respectively) were considered by the investigator to be causally related to the study drug. The patient who experienced septic shock as a TEAE had received 1 dose of the study drug in cohort 2. This patient died, and the cause of death was listed as severe sepsis, septic shock secondary to urinary tract infection, and severe neutropenia. It should be noted that this patient had been hospitalized with sepsis and a urinary tract infection just 5 days before study enrollment.

### Cardiac Safety Evaluations

A comparison of echocardiographic parameters at the time of screening and study completion revealed no clinically significant changes in any cohort for any echocardiographic parameter over the course of the study. Cardiac safety evaluations demonstrated that no patients experienced a decrease in left ventricular ejection fraction <45% of predicted. One patient in the group treated with a dose of 450 mg/m<sup>2</sup> experienced a prolongation in the QTc interval to >500 milliseconds but was withdrawn from the study after the first cycle because of liver failure secondary to disease. Seven patients (28%) had elevated serum troponin levels ( $\geq 0.016$  ng/mL or  $\geq 1.5$  times the upper limit of normal) at 1 or more time points during the study, including 1 patient with elevated levels at the time of screening but not during the study, and 6 with elevated levels (range, 0.016-0.30 ng/mL) during the study. All incidences of increased serum troponin observed during the study either were deemed not clinically significant ( $\geq 5$  times the upper limit of normal) or were not assessed for clinical significance.

### Tumor Response Evaluations

Best overall responses in the total population are summarized in Table 4. The overall response rate was 20%, including a PR rate of 20% and a CR rate of 0%. The

**TABLE 3.** Incidences of Study Drug-Related TEAEs (All Grades) Occurring in  $\geq 10\%$  of the Total Population, or Grade 3/4 Adverse Events Occurring in Any Patient

| Adverse Event, No. (%)    | Initial Aldoxorubicin Dose Cohort |        |   |         |  |         |                          |         |
|---------------------------|-----------------------------------|--------|---|---------|--|---------|--------------------------|---------|
|                           | 230 mg/m <sup>2</sup><br>(n = 5)  |        | 350 mg/m <sup>2</sup><br>(n = 18 <sup>a</sup> ) |         | 450 mg/m <sup>2</sup><br>(n = 2 <sup>a</sup> ) |         | All Patients<br>(N = 25) |         |
|                           | All                               | 3/4    | All   | 3/4     | All  | 3/4     | All                      | 3/4     |
| <b>Grade</b>              |                                   |        |   |         |  |         |                          |         |
| <b>Hematologic</b>        |                                   |        |   |         |  |         |                          |         |
| Neutropenia               | 2 (40)                            | —      | 18 (100)  | 16 (89) | 2 (100)  | 2 (100) | 22 (88)                  | 18 (72) |
| Thrombocytopenia          | 2 (40)                            | 1 (20) | 16 (89)   | 6 (33)  | 2 (100)  | 1 (50)  | 20 (80)                  | 8 (32)  |
| Anemia                    | 3 (60)                            | 1 (20) | 13 (72)   | 4 (22)  | 2 (100)  | 1 (50)  | 18 (72)                  | 6 (24)  |
| Febrile neutropenia       | —                                 | —      | 2 (11)  | 2 (11)  | 2 (100)  | 2 (100) | 4 (16)                   | 4 (16)  |
| <b>Nonhematologic</b>     |                                   |        |   |         |  |         |                          |         |
| Nausea                    | 4 (80)                            | —      | 13 (72)   | —       | 2 (100)  | —       | 19 (76)                  | —       |
| Fatigue                   | 1 (20)                            | 1 (20) | 12 (67)   | 2 (11)  | 1 (50)   | —       | 14 (56)                  | 3 (12)  |
| Alopecia                  | —                                 | —      | 12 (67)   | —       | 1 (50)   | —       | 13 (52)                  | —       |
| Stomatitis                | 1 (20)                            | —      | 10 (56)   | —       | 2 (100)  | 2 (100) | 13 (52)                  | 2 (8)   |
| Vomiting                  | 2 (40)                            | —      | 6 (33)  | —       | 1 (50)   | —       | 9 (36)                   | —       |
| Oropharyngeal pain        | 1 (20)                            | —      | 5 (28)  | —       | —  | —       | 6 (24)                   | —       |
| Decreased appetite        | —                                 | —      | 5 (28)  | —       | 1 (50)   | —       | 6 (24)                   | —       |
| Mouth ulceration          | —                                 | —      | 3 (17)  | —       | 1 (50)   | —       | 4 (16)                   | —       |
| Constipation              | —                                 | —      | 2 (11)  | —       | 1 (50)   | —       | 3 (12)                   | —       |
| Dysphagia                 | —                                 | —      | 2 (11)  | —       | 1 (50)   | —       | 3 (12)                   | —       |
| Asthenia                  | —                                 | —      | 2 (11)  | 1 (6)   | —  | —       | 2 (8)                    | 1 (4)   |
| Dehydration               | —                                 | —      | 2 (11)  | 1 (6)   | —  | —       | 2 (8)                    | 1 (4)   |
| GERD                      | 1 (20)                            | 1 (20) | —   | —       | —  | —       | 1 (4)                    | 1 (4)   |
| Oral candidiasis          | —                                 | —      | 1 (6)   | 1 (6)   | —  | —       | 1 (4)                    | 1 (4)   |
| Sepsis                    | —                                 | —      | 1 (6)   | 1 (6)   | —  | —       | 1 (4)                    | 1 (4)   |
| Septic shock <sup>b</sup> | —                                 | —      | 1 (6)   | 1 (6)   | —  | —       | 1 (4)                    | 1 (4)   |

Abbreviations: GERD, gastroesophageal reflux disease; TEAE, treatment-emergent adverse event.

<sup>a</sup>One patient initially received a dose of 450 mg/m<sup>2</sup> in cycle 1 and subsequently received a dose of 350 mg/m<sup>2</sup> in cycles 2 through 8.

<sup>b</sup>Septic shock that resulted in death (grade 5) occurred in one patient.

patient in cohort 3 who had a PR achieved this response after 1 cycle at a dose of 450 mg/m<sup>2</sup> and 5 cycles at the dose level of 350 mg/m<sup>2</sup>. The median PFS for the total population was 4.80 months (Fig. 2A), and the median OS was 11.25 months (Fig. 2B).

Best responses for the 13 patients with soft tissue sarcoma in cohort 2 who received aldoxorubicin at the MTD are summarized in Table 5 and Figure 3. Twelve of these 13 patients had received prior chemotherapy, including 7 patients who had received prior doxorubicin, epirubicin, and/or pegylated liposomal doxorubicin and had experienced either no response or disease progression on prior therapy. Of 8 patients with evidence of tumor shrinkage while receiving aldoxorubicin, 5 had exhibited no objective response to prior anthracycline therapy (Fig. 3). During treatment with aldoxorubicin, 9 of the 13 patients had achieved either a PR or SD lasting at least 4 months. For these 13 patients, the median PFS was 11.25 months (Fig. 2C) and the median OS was 21.71 months (Fig. 2D).

## DISCUSSION

This phase 1B/2 study of a modified formulation of aldoxorubicin in patients with advanced solid tumors

established the MTD of aldoxorubicin as 350 mg/m<sup>2</sup> (equivalent to 260 mg/m<sup>2</sup> doxorubicin) administered every 3 weeks for up to 8 cycles. At this dose and administration schedule of aldoxorubicin, cumulative doses of >2000 mg/m<sup>2</sup> of doxorubicin equivalents have been achieved, which is >3.5 times the peak cumulative dose of standard doxorubicin, with no evidence of clinically significant acute cardiac abnormalities. These observations suggest that therapy with aldoxorubicin could allow for much higher doxorubicin dose equivalents to be used in the treatment of patients with cancer, either alone or as part of high-dose chemotherapy combinations, without a commensurate increase in acute cardiotoxicity. To the best of our knowledge, the longer-term effects of aldoxorubicin on cardiotoxicity are unknown at this time.

Major grade 3/4 hematologic AEs reported with aldoxorubicin dosing included neutropenia and thrombocytopenia that generally resolved before the start of the next cycle. We hypothesize that the high frequency of hematologic AEs may be attributable to the release of doxorubicin from aldoxorubicin in the relatively acidic environment of the bone marrow, although the current study was not designed to examine that hypothesis.

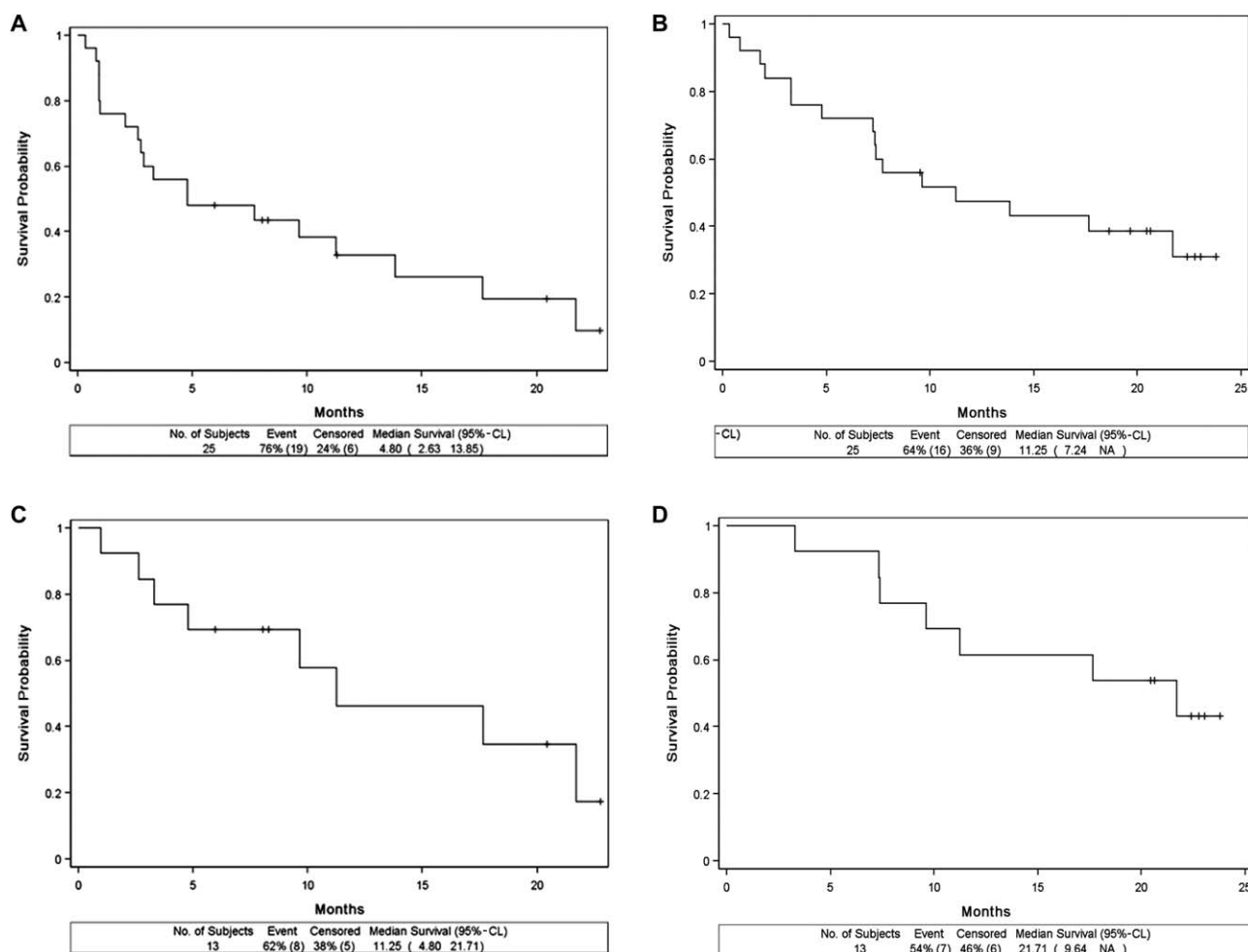
**TABLE 4.** Best Overall Response in the Tumor Response-Evaluable Population

| Best Response, No. (%)     | Initial Aldoxorubicin Dose Cohort |   |  |                          |
|----------------------------|-----------------------------------|---|--|--------------------------|
|                            | 230 mg/m <sup>2</sup><br>(n = 5)  | 350 mg/m <sup>2</sup><br>(n = 18 <sup>a</sup> ) | 450 mg/m <sup>2</sup><br>(n = 2 <sup>a</sup> ) | All patients<br>(N = 25) |
| CR                         | —                                 | —   | —  | —                        |
| PR                         | —                                 | 4 (22)  | 1 (50)   | 5 (20)                   |
| Overall response (CR + PR) | —                                 | 4 (22)  | 1 (50)   | 5 (20)                   |
| SD                         | 1 (20)                            | 9 (50)  | —  | 10 (40)                  |
| PD                         | 2 (40)                            | 3 (17)  | —  | 5 (20)                   |
| Not done <sup>b</sup>      | 2 (40)                            | 2 (11)  | 1 (50)   | 5 (20)                   |
| Median PFS (95% CL), mo    | —                                 | —   | —  | 4.80 (2.63-13.85)        |

Abbreviations: 95% CL, 95% confidence level; CR, complete response; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

<sup>a</sup>One patient initially received a dose of 450 mg/m<sup>2</sup> in cycle 1 and subsequently received a dose of 350 mg/m<sup>2</sup> in cycles 2 through 8. This patient achieved a PR while receiving the lower dose.

<sup>b</sup>Response was not assessed in these patients because of death or withdrawal from the study before or at the time of the first assessment on day 28.



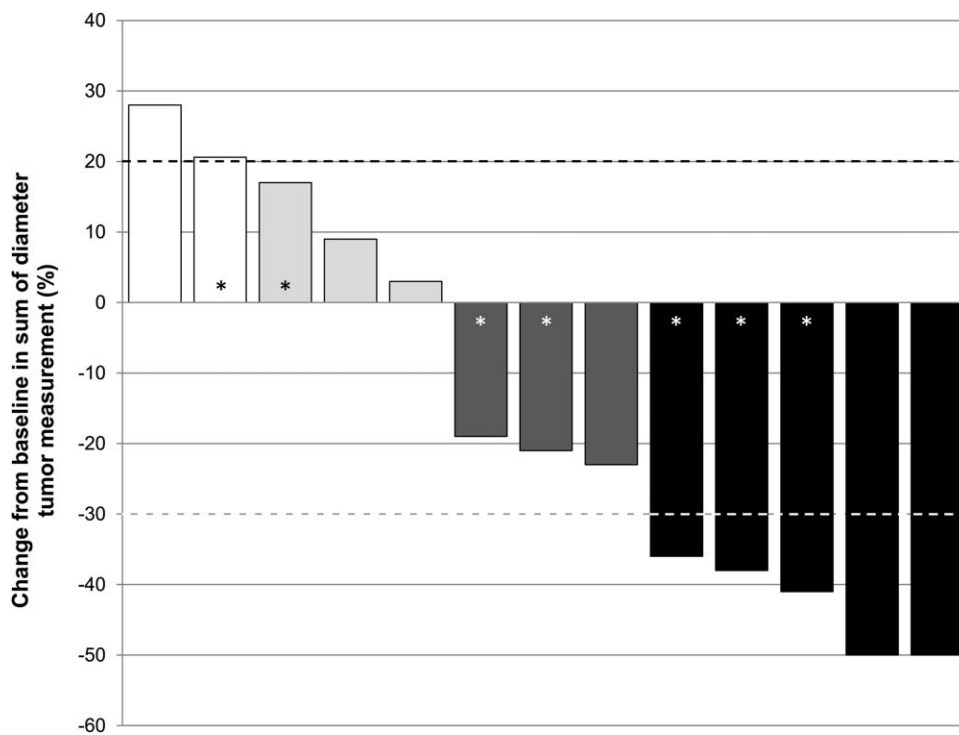
**Figure 2.** Kaplan-Meier estimates of (A) progression-free survival and (B) overall survival are shown for the total population (25 patients) and (C) progression-free survival and (D) overall survival are shown for those patients with soft tissue sarcoma who were treated with aldoxorubicin at a dose of 350 mg/m<sup>2</sup> (13 patients; this includes 1 patient who initially received a dose of 450 mg/m<sup>2</sup> in cycle 1 and subsequently received a dose of 350 mg/m<sup>2</sup> in cycles 2 through 8). 95% CL indicates 95% confidence level; NA, not applicable.

**TABLE 5.** Best Response of Patients With Soft Tissue Sarcoma in the Cohort Treated at a Dose of 350 mg/m<sup>2</sup>

| Best Response, No. (%)  | Aldoxorubicin, 350 mg/m <sup>2</sup><br>(N = 13 <sup>a</sup> ) | Histologic Subtype  |
|-------------------------|--|---|
| CR                      | —  | —   |
| PR                      | 5 (38)   | 1 each: liposarcoma, leiomyosarcoma, spindle cell sarcoma, pleomorphic sarcoma, and malignant peripheral nerve sheath tumor |
| SD                      | 6 (46)   | All: leiomyosarcoma   |
| PD                      | 2 (15)   | 1 each: leiomyosarcoma and hemangiopericytoma   |
| Median PFS (95% CL), mo | 11.25 (4.80-21.71)   |   |

Abbreviations: 95% CL, 95% confidence level; CR, complete response; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

<sup>a</sup>Includes one patient who initially received a dose of 450 mg/m<sup>2</sup> in cycle 1 and subsequently received a dose of 350 mg/m<sup>2</sup> in cycles 2 through 8.



**Figure 3.** Waterfall plot of best response is shown for the patients with soft tissue sarcoma who were treated with aldoxorubicin at a dose of 350 mg/m<sup>2</sup> (maximum tolerated dose; 13 patients). Asterisk indicates those patients who had received prior therapy with doxorubicin, epirubicin, or pegylated liposomal doxorubicin.

Frequent nonhematologic AEs included nausea, fatigue, alopecia, stomatitis, vomiting, and oropharyngeal pain. The majority of study drug-related, nonhematologic TEAEs occurring in any cohort were mild to moderate in severity (grade 1/2). Compared with native doxorubicin, treatment with aldoxorubicin did not result in any unexpected AEs. It is of considerable importance to note that no clinically significant cardiac toxicities were observed with aldoxorubicin treatment in the current study. Safety findings with the present formulation of aldoxorubicin

appear to compare favorably with those reported in the previous phase 1 study.<sup>18</sup>

All 25 patients in the current study were evaluable for tumor response. Among the total population, the ORR was 20%, the SD rate was 40%, and the overall disease control rate was 60%. Among patients with soft tissue sarcoma who were treated in cohort 2 at the MTD (13 patients), the ORR was 38% and the SD rate was 46%. Of these 13 patients, 9 (69%) had a PR or SD lasting ≥4 months. The median PFS was 11.25 months for this



cohort of patients with soft tissue sarcoma. The preliminary efficacy of aldoxorubicin shown in the current study in patients with soft tissue sarcoma, the majority of whom had either failed to respond or developed disease recurrence after prior chemotherapies, exceeded prior efficacy findings of single-agent anthracycline in similar populations, in which response rates have been reported to range from 12% to 23%.<sup>20-22</sup> However, it should be noted that the current study involved only a single site.

Targeted delivery of anticancer agents is a highly sought-after goal of cancer therapy. Albumin is viewed as a tumor-selective drug carrier because serum albumin accumulates in tumor tissue,<sup>23</sup> possibly as a source of energy and nutrition for tumor growth.<sup>24</sup> The pathophysiology of tumor tissue, characterized by hypervascularization, enhanced vascular permeability, and impaired lymphatic drainage, allows macromolecules such as serum albumin to accumulate passively in tumor tissue and become retained.

Nanoparticle albumin-bound (“nab”) technology has already proven effective as a means to deliver chemotherapeutic agents. For example, nab-paclitaxel, which is currently approved for the treatment of patients with metastatic breast cancer, locally advanced or metastatic non-small cell lung cancer, and metastatic pancreatic cancer,<sup>25</sup> can be administered at an MTD of 300 mg/m<sup>2</sup>, which is approximately twice the usual dose range of paclitaxel (135-200 mg/m<sup>2</sup>).<sup>26</sup> Although nab-chemotherapy can be effective, the use of circulating albumin as a drug carrier may have certain advantages over synthesized drug-albumin conjugates or complexes.<sup>15</sup> First, this precludes the need for commercial albumin, which may keep the cost of drug manufacturing relatively lower. Second, aldoxorubicin binds covalently to albumin in the bloodstream and is not released unless in an acidic environment. In contrast, albumin in nab-chemotherapy is not covalently linked to nanoparticles and can dissociate at the time of infusion. Third, the organic chemistry principles applied to generate albumin-binding drugs are straightforward, thereby highlighting the potential to apply these principles to generate albumin-binding versions of an array of drugs. Finally, the pharmaceutical products of albumin-binding drugs can be analyzed with comparable ease.

The results of the current study have demonstrated that aldoxorubicin is reasonably safe when administered at a dose of 350 mg/m<sup>2</sup> as a 30-minute intravenous infusion on day 1 of each 21-day cycle for up to 8 consecutive cycles. Aldoxorubicin is currently under investigation in larger, prospectively designed clinical studies, particularly

those involving patients with soft tissue sarcoma or other anthracycline-sensitive cancers.

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## CONFLICT OF INTEREST DISCLOSURES

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