16. Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer* 1957, **11**, 359–377.
17. WHO. Histological typing of breast tumors. International histological classification of tumors No 2. 2nd ed. Geneva World Health Organisation, 1981.
18. Baak JPA, Oort J. Obtaining quantitative data. In: Baak JPA, Oort J, eds. *A Manual of Morphometry in Diagnostic Pathology*. Berlin, Springer, 1983, 15–26.
19. Haapasalo H, Collan Y, Pesonen E. Volume-corrected mitotic index (M/V index) – the standard of mitotic activity in neoplasms. *Path Res Pract* 1989, **185**, 551–554.
20. Lee E, Desu M. A computer program for comparing k samples with right censored data. *Comput Programs Biomed* 1972, **2**, 315–318.
21. Cox DR. Regression models and life tables with discussion. *J R. Stat Soc B* 1972, **34**, 187–192.
22. Aaltomaa S, Lipponen P, Eskelinen M, Alhava E, Syrjänen K. Nuclear morphometry and mitotic indexes as prognostic factors in breast cancer. *Eur J Surg* 1991, **157**, 319–324.
23. Baak JPA, Van Dop H, Hermans J. The value of morphometry to classic prognosticators in breast cancer. *Cancer* 1985, **56**, 374–382.
24. Eskelinen M, Collan Y, Pajarinen P, Pesonen E, Kettunen K, Nordling S. An improved prognostic index of axillary node involvement in breast cancer incorporating DNA ploidy and tumour size. *Acta Chir Scand* 1990, **156**, 521–527.
25. Eskelinen MJ, Pajarinen P, Collan Y, Pesonen E, Alhava E, Kettunen K, Nordling S. Relationship between DNA ploidy and survival in patients with primary breast cancer. *Br J Surg* 1989, **76**, 830–834.
26. Toikkanen S, Joensuu H, Klemi P. Prognostic significance of nuclear DNA content in invasive breast cancer—a study with long-term follow-up. *Br J Cancer* 1989, **60**, 693–700.
27. Uyterlinde AM, Baak JPA, Schipper NW, Peterse H, Matze E, Meijer CJ. Further evaluation of the prognostic value of morphometric and flow cytometric parameters in breast cancer patients with long follow-up. *Int J Cancer* 1990, **45**, 1–7.
28. Reilly SM, Camplejohn RS, Barnes DM, Millis RR, Rubens RD, Richards MA. Node negative breast cancer: prognostic subgroups defined by tumor size and flow cytometry. *J Clin Oncol* 1990, **8**, 2040–2046.
29. Alanko A, Heinonen E, Scheinin T, Tolppanen E-M, Vihko R. Significance of estrogen and progesterone receptors, disease-free interval, and site of first metastasis on survival of breast cancer patients. *Cancer* 1985, **56**, 1696–1700.
30. Dykins R, Corbett IP, Henry JA, *et al.* Long-term survival in breast cancer related to overexpression of the c-*erb* B-2 oncoprotein: an immunohistochemical study using monoclinal antibody NCL-CB11. *J Pathol* 1991, **163**, 105–110.
31. Rilke F, Colnaghi MI, Cascinelli N, *et al.* Prognostic significance of HER-2/NEU expression in breast cancer and it's relationship to other prognostic factors. *Int J Cancer* 1991, **49**, 44–49.
32. Tubiana M, Pejovic MH. Chavaudra N, Contesso G, Malaise EP. The long term prognostic significance of the thymidine labeling index in breast cancer. *Int J Cancer* 1984, **33**, 441–445.
33. Tubiana M, Pejovic MJ, Contesso G, Chavaudra N, Contesso G, Malaise EP. Kinetic parameters and the course of the disease in breast cancer. *Cancer* 1981, **47**, 937–943.

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Suramin in Advanced Platinum-resistant Ovarian Cancer

Eddie Reed, Michael R. Cooper, Renato V. LaRocca, Frieda Bostick-Bruton and Charles E. Myers

10 patients with ovarian cancer, whose disease had progressed while receiving platinum-based therapy, were entered onto a phase II clinical trial of the antiproliferative agent suramin. Suramin was administered in a fashion that is associated with durable objective disease response in patients with hormonally resistant metastatic prostate cancer. No individual had an objective response to therapy in this study, but 3 of 9 evaluable patients (33%) experienced disease stabilisation and subjective clinical improvement for periods ranging from 2 to 5 months. Disease stabilisation was associated with prolonged periods of comparatively high plasma levels of drug, which appeared to be determined primarily by reduced drug clearance. We conclude that suramin has potential activity in platinum-resistant ovarian cancer, and we have initiated a second clinical trial using pharmacological information derived from this study.
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INTRODUCTION

A NUMBER of recent clinical trials have attempted to identify chemotherapeutic agents that might cause disease regression in recurrent ovarian cancer that is resistant to cisplatin. Taxol [1], hexamethylmelamine [2], 5-fluorouracil and mitomycin C [3], and ifosfamide [4] are among the regimens reported to have activity in platinum-resistant disease. With the exception of taxol, the author's precise definition of platinum-resistance cannot be clearly discerned in any of these studies. Further, these cytotoxic agents have profound toxicity and are usually associated with disease responses of short duration.

Recent clinical studies show that suramin, a novel anticancer agent which appears to act primarily through antiproliferative mechanisms, is active against advanced stage prostate cancer, adrenocortical cancer, and some types of refractory lymphomas [5–7]. In addition, preclinical data from our group and from the

group of Alberts *et al.* [8], show that suramin has substantial *in vitro* activity against ovarian cancer cell lines. Because of the recent success in several diseases and encouraging *in vitro* activity, we undertook a clinical study to assess the potential of suramin in platinum-resistant ovarian cancer.

PATIENTS AND METHODS

Clinical studies

Patients with advanced stage ovarian cancer who experienced progressive disease on their initial cisplatin-based treatment regimen or on 'high dose' carboplatin therapy [9] were treated with suramin. Progressive disease was defined as > 25% growth of tumour bulk while receiving therapy. Patients were entered onto an approved phase II experimental clinical treatment protocol in the Medicine Branch of the National Cancer Institute. All patients were treated in the Clinical Center of the National Institutes of Health (Bethesda, Maryland).

Suramin was administered in a fashion that has been previously shown to have activity in advanced stage prostate cancer [7]. Suramin was initially given in a dose of 350 mg/2/day by continuous intravenous infusion for 7 days. Since suramin is associated with adrenal gland suppression, corticoids were administered concurrently, which included hydrocortisone, 20 mg every morning and 10 mg every evening. Blood levels of suramin were measured at the end of the 7 day infusion and weekly thereafter. The objective of this approach was to achieve a plasma concentration of ~300 μg/ml by the end of this second week of drug administration, at which time the drug infusion was stopped. Calculation of the drug infusion rate for week 2 was based upon an empirically derived nomogram. The patient's plasma level of drug was measured weekly or bi-weekly, and subsequent doses of drug were given when the plasma level fell below 100 μg/ml.

Toxicities were assessed according to standard NCI-CTEP Common Toxicity Criteria. Patients were considered to have progressive disease when there was a ≥ 25% increase in the sum of the products of the perpendicular diameters of all measurable lesions. In this report, all patients who were assessed as having stable disease experienced all of the following: (a) subjective clinical improvement on therapy; (b) a greater than 50% decline in serum CA-125 level; and (c) a reduction in tumour mass which was less than a 50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions. Patients were assessed for response on a monthly basis.

Pharmacokinetic assessments and statistical evaluations

To assess pharmacokinetic parameters in this patient population, we used a two-compartment pharmacokinetic model which was constructed for suramin treatment of prostate cancer patients [10]. Bayesian fitting was performed using a pharmacokinetic program provided by Abbott Laboratories. Comparison of stable disease and progressive disease patients was done using the exact Wilcoxon test [11]. All P values given are two-sided.

Correspondence to E. Reed.
E. Reed and F. Bostick-Bruton are at the Medicine Branch; and M.R. Cooper, R.V. LaRocca and C.E. Myers are at the Clinical Pharmacology Branch, National Cancer Institute, Building 10, Room 12N226, Bethesda, Maryland 20892, U.S.A.
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Table 1. Patients' characteristics

Patient No.	Age	Extra-abdominal disease site	Disease response	No. of prior regimens
1	46	Left perinephric mass	Not evaluable for response	4
2	45	Abdominal wall	Progressive disease	1
3	75	None	Progressive disease	5
4	22	None	Stable disease for 5 months	3
5	55	None	Progressive disease	1
6	55	None	Progressive disease	1
7	63	Skin	Progressive disease	4
8	48	None	Stable disease for 4 months	4
9	31	Extra-abdominal lymph nodes	Stable disease for 2 months	1
10	57	Vaginal wall	Progressive disease	3

RESULTS

Patients' characteristics

Patients were eligible for study who had advanced stage ovarian cancer of epithelial histology, which had progressed on their initial treatment regimen containing cisplatin or progressed on high dose carboplatin therapy. All patients had a Karnofsky performance status of 70 or better. Table 1 summarises the most prominent features of the 10 patients studied. Age ranged from 22 to 75 years; 5 of 10 patients had extra-abdominal involvement of disease at the initiation of therapy; and the number of prior regimens ranged from 1 to 5.

Of 9 evaluable patients, 3 had stable disease for periods ranging from 2 to 5 months. In the 3 patients with stable disease, CA-125 levels (U/ml) dropped from 1092 to 418, from 199 to 70, and from 156 to 36, respectively. Patient number 1 is evaluable for toxicity but not evaluable for response because of non-cancer-related death. This patient had shown clinical improvement at the clinical visit just prior to death.

Suramin pharmacology in stable and progressive disease patients

After the tenth patient was entered onto the study, we became aware of differences between patients with stable disease versus patients with progressive disease, with respect to suramin blood levels. Patients with stable disease maintained levels of drug above 150 μg/ml for at least 30 days, and above 100 μg/ml for at least 60 days. In contrast, all patients who had progressive disease had plasma drug measurements that fell below these levels at the respective time points.

We immediately became concerned whether suramin dosing or renal function may have been substantively different in these two patient groups. Peak suramin levels obtained did not differ between individuals with stable [mean (S.D.) 295 (11) μg/ml] versus progressive [268 (29) μg/ml] disease; and values for 24 h creatinine clearance were the same for those patients with stable disease [69 (27) ml/min] as those with progressive disease [63 (20) ml/min].

Drug pharmacokinetics were assessed in each patient using a Bayesian analysis based on a two-compartment model. The

Table 2. Summary of pharmacokinetic evaluation

	Stable disease (n = 3)	Progressive disease (n = 6)	P value
Initial volume of distribution (l/kg)	0.171	0.164	N.S.
Initial clearance (ml/h/kg)	0.235	0.340	0.12
Intercompartmental rate constants (ml/h)			
k_{12}	5.63	5.42	N.S.
k_{21}	2.97	2.98	N.S.
Total clearance (l/h)	0.16	0.21	0.29
Rate constants for drug elimination from the body (ml/h)			
k_{10} (total)	1.37	2.18	0.025
alpha phase	9.55	9.93	N.S.
beta phase	0.433	0.634	0.050
Drug half-life (h)	1633	1138	0.033

N.S. = not significant.

summary analysis of this comparison is shown in Table 2. When grouped by stable versus progressive disease, there was no difference between patient groups in the initial volume of drug distribution nor the intercompartmental rate constants K12 or K21. There were trends suggesting that initial drug clearance from the body was more rapid in patients with progressive disease, as was true for the total clearance values. There were statistically significant differences between groups showing that total drug elimination from the body was more rapid in patients with progressive disease (the K10, P = 0.025.), and that the drug half-life was markedly shortened in patients with progressive disease (P = 0.033). The difference between the two groups in the K10 appears to be dominated by the beta phase of elimination (P = 0.050).

Suramin related toxicities

Generally, the drug was well tolerated. 3 patients experienced clinical toxicities of NCI-CTEP grade 4 (2 patients, infection/sepsis; 1 patient, rash). No patient experienced the severe type of peripheral neuropathy that was seen in early clinical studies of this agent [12]. Grade 4 haematopoetic toxicities seen with this regimen, including lymphocytopenia (6 patients), thrombocytopenia (2 patients), and granulocytopenia (1 patient). Non-haematopoetic grade 2–3 laboratory toxicities included renal dysfunction and liver enzyme abnormalities, none of which resulted in substantial clinical toxicity and were readily reversible.

DISCUSSION

Suramin is a novel anticancer agent, which may act through mechanisms which may be interpreted as antiproliferative, but not necessarily cytotoxic, in nature. In our limited clinical investigation, suramin appeared to stabilise the disease in 3 of 9 evaluable patients (33%), each of whom had experienced progressive disease while receiving platinum-based chemotherapy. Clinically, suramin appeared to effect an antiproliferative tumour state in patients whose disease stabilised, but did not appear to effect active cytotoxicity. The 3 patients with stable disease were those who maintained high blood levels of drug for prolonged periods of time. This was a result of reduced drug elimination from the body, in a setting where renal function appeared to be the same in the two groups. Unfortunately, we did not perform measurements of free drug in these patients, nor did we measure drug levels in third-space fluids.

Clinical studies conducted by Armand and colleagues demonstrate substantial suramin activity in several malignant diseases and that the precise mode of drug administration may be an important variable in the induction of disease response [13]. This observation is confirmed in a preliminary report from Alberts *et al.* in a study of platinum-resistant ovarian cancer patients [8]. The findings we present here are consistent with these reports.

Administered in the fashion described above, suramin was well tolerated, suggesting the possibility that prolonged patient exposure to drug levels in the range of 100 to 300 μg/ml may be clinically achievable. Given the current evidence for potential clinical activity of this agent and its association with good patient tolerance, we have initiated a study designed to maintain patients at high plasma levels of drug for prolonged times. We believe that this approach warrants further study.

1. McGuire WP, Rowinsky EK, Rosenshein NB, *et al.* Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. *Ann Intern Med* 1989, **111**, 273–279.
2. Manetta A, MacNeill C, Lyter JA, *et al.* Hexamethylmelamine as a single second-line agent in ovarian cancer. *Gynecol Oncol* 1990, **36**, 93–96.
3. Alberts DS, Garcia-Kendall D, Surwit EA. Phase II trial of mitomycin C plus 5-FU in the treatment of drug-refractory ovarian cancer. *Semin Oncol* 1988, **15** (Suppl 4), 22–26.
4. Sutton GP, Blessing JA, Photopulos G, Berman ML, Homesley HD. Early phase II Gynecologic Oncology Group experience with ifosfamide/mesna in gynecologic malignancies. *Cancer Chemother Pharmacol* 1990, **26**, Suppl: S55–S58.
5. Stein C, LaRocca R, Myers C. Suramin: an old compound with new biology. *PPO Updates* 1990, **4**, 1–12.
6. LaRocca R, Stein C, Myers C. Suramin: prototype of a new generation of antitumor compounds. *Cancer Cells* 1990, **2**, 106–115.
7. Stein CA, LaRocca RV, Thomas R, McAtee N, Myers CE. Suramin: an anticancer drug with a unique mechanism of action. *J Clin Oncol* 1989, **7**, 499–508.
8. Alberts D, Miranda E, Dorr R, *et al.* Phase II and pharmacokinetic and human tumor cloning assay study of suramin in advanced ovarian cancer. *Proc Am Soc Clin Oncol* 1991, **10**, 187, abst No. 609.
9. Reed E, Janik J, Bookman MA, *et al.* High dose carboplatin and rGM-CSF in advanced stage refractory ovarian cancer. *Proc ASCO* 1990, **9**, 157, abst No. 609.
10. Lieberman R, Katzper M, Cooper M, *et al.* NONMEM population pharmacokinetic analysis and Bayesian forecasting during suramin therapy in prostate cancer: one- versus two-compartment PK models. *Proc ASCO* 1990, **9**, 68, Abst No. 262.
11. Armitage P. *Statistical Methods in Medical Research.* Oxford, Blackwell Scientific Publications, 1971.
12. LaRocca R, Meer J, Gilliatt RW, *et al.* Suramin-induced polyneuropathy. *Neurology* 1990, **40**, 954–960.
13. Armand JP, Bonnay M, Gandia D, *et al.* Phase I–II suramin study in advanced cancer patients. *Proc Am Asso Cancer Res* 1991, **32**, 175, abst No. 1042.