

Survival Efficacy of Adjuvant Cytosine-Analogue CS-682 in a Fluorescent Orthotopic Model of Human Pancreatic Cancer

Matthew H. Katz,¹ Michael Bouvet,¹ Shinako Takimoto,¹ Daniel Spivack,¹ Abdool R. Moossa,¹ and Robert M. Hoffman^{1,2}

¹Department of Surgery, University of California at San Diego, San Diego, California, and ²AntiCancer, Inc., San Diego, California

ABSTRACT

Adjuvant treatment with the cytosine analogue 1-(2-C-cyano-2-deoxy- β -D-arabino-pentofuranosyl)-N⁴-palmitoylcytosine (CS-682) results in a highly significant increase in survival in the aggressive orthotopic MIA-PaCa-2 human pancreatic cancer mouse model. Seven days after implantation, mice were randomized into eight groups, depending on whether they were to be treated by tumor resection, 5 weeks of CS-682 chemotherapy at 40–60 mg/kg once daily, or both. Throughout the course of treatment, noninvasive optical whole-body imaging based on brilliant red fluorescent protein expression of the tumor permitted visualization and quantification of primary, metastatic, and recurrent disease. Total tumor burden negatively correlated with survival. Untreated mice died of disseminated disease with a median survival of 26 days. Surgical resection alone conferred a small but significant survival advantage (median survival, 28 days, $P = 0.03$). Primary CS-682 treatment at all doses also significantly prolonged survival compared with untreated animals ($P < 0.05$) and was more effective than surgery alone at doses of 50 and 60 mg/kg (median survival, 34 days, $P = 0.045$, and 38.5 days, $P = 0.03$, respectively). Maximal survival (median, 48 days, with 30% of animals surviving longer than 60 days) was achieved by adjuvant CS-682 (50 mg/kg), given after surgical resection of the primary pancreatic tumor ($P = 0.004$ compared with surgery alone). The results demonstrate that adjuvant oral administration of CS-682 for pancreatic cancer is highly effective with acceptable toxicity, suggesting its potential for cure of this disease in appropriate combinations.

INTRODUCTION

The cornerstone of management for patients with localized pancreatic cancer is operative tumor resection by pancreaticoduodenectomy or distal pancreatectomy, with complete resection of disease offering the only chance for cure. Advances in surgical technique and postoperative care over the past three decades have resulted in low rates of associated perioperative morbidity and mortality. Various studies have confirmed an acceptable postoperative quality of life that legitimizes the use of these demanding surgical procedures (1, 2). Nonetheless, even in experienced, high-volume centers, median survival for patients with pancreatic ductal adenocarcinoma after operative resection remains under 21 months (3, 4), largely due to a high rate of recurrent disease in both the pancreatic bed and distant sites. Even in the subset of patients in whom a curative resection has been successfully accomplished—one in which all surgical margins are grossly and microscopically negative—rates of treatment failure approach 70% (5, 6). Notably, attempts to reduce recurrence and prolong survival with more radical surgical procedures have not been successful (7, 8).

The use of adjuvant chemotherapy or chemoradiotherapy in the

treatment of solid malignancies rests on the concept that tumor recurrence after surgery is due to subclinical, microscopic foci of residual disease. By eliminating such occult disease, the early application of cytotoxic agents may prolong survival after resection. Protocols exploiting this strategy have proven to be important components of the therapy of many solid tumors and are now routine for treatment of cancers of the colon (9) and the breast (10). Unfortunately, the benefit of adjuvant therapies in the treatment of pancreatic cancer has yet to be definitively demonstrated. Nonetheless, accumulating experience with the well-known agent 5-fluorouracil suggests that a role may exist for adjuvant chemotherapy, either alone or in combination with radiation (11, 12). Additional studies exploring the postoperative use of other cytotoxic agents, including the promising deoxycytidine analogue gemcitabine, are currently in progress (3).

We recently described the ability of a novel, orally administered cytosine analogue, 1-(2-C-cyano-2-deoxy- β -D-arabino-pentofuranosyl)-N⁴-palmitoylcytosine (CS-682), to inhibit the growth and development of human pancreatic ductal adenocarcinoma in a red fluorescent, orthotopic model (13). In particular, we demonstrated that the efficacy of this agent may be attributed in large part to its ability to inhibit the development of local and distant tumor metastases. On the basis of these findings, we postulated that adjuvant use of this agent may be effective in destroying microscopic tumor foci left in the abdomen after attempted surgical resection, thereby preventing tumor recurrence and prolonging survival.

In this study, we report the efficacy of oral CS-682 in the adjuvant treatment of metastatic pancreatic cancer and clearly demonstrate its ability to prolong survival compared with no treatment, primary use of the agent, and surgical resection alone. In this study, we used a highly aggressive clone of the human pancreatic cancer cell line MIA-PaCa-2 that we have engineered to selectively express high levels of the *Discosoma* red fluorescent protein (RFP; Ref. 14, 15). This brightly fluorescent model allows us to visualize and to quantify tumor burden noninvasively throughout the course of treatment, permitting real-time analysis and comparison of therapeutic interventions.

MATERIALS AND METHODS

Cell Line. The MIA-PaCa-2 pancreatic cancer cell line was obtained from the American Type Culture Collection (Manassas, VA). Cells were maintained in DMEM media supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin and streptomycin (Life Technologies, Inc., Grand Island, NY). Cells were cultured at 37°C in a 5% CO₂ incubator.

RFP Retroviral Transduction and Selection of MIA-PaCa-2-RFP Pancreatic Cancer Cells. The pDsRed-2 vector (Clontech Laboratories, Inc., Palo Alto, CA) was used to engineer MIA-PaCa-2 clones stably expressing RFP. This vector expresses RFP and the neomycin resistance gene on the same bicistronic message and has been demonstrated to exhibit low toxicity in mammalian cell lines. pDsRed-2 was produced in PT67-packaging cells. RFP transduction was initiated by incubating 20% confluent MIA-PaCa-2 cells with retroviral supernatants of the packaging cells and DMEM for 24 h. Fresh medium was replenished at this time, and cells were allowed to grow in the absence of retrovirus for 12 h. This procedure was repeated until high levels of RFP expression, as determined using fluorescence microscopy, were achieved. Cells were then harvested by trypsin/EDTA and subcultured into selective

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Requests for reprints: Robert M. Hoffman, AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111. Phone: (858) 654-2555; Fax: (858) 268-4175; E-mail: all@anticancer.com.

medium that contained 200 $\mu\text{g}/\text{ml}$ G418. The level of G418 was increased to 2000 $\mu\text{g}/\text{ml}$ stepwise. Clones expressing high levels of RFP were isolated with cloning cylinders as needed and were amplified and transferred using conventional culture methods. High RFP-expression clones were isolated in the absence of G418 for 10 passages to select for stable expression of RFP *in vivo*.

Animals. Male nude mice (NCR-nu) between 4 and 6 weeks of age were maintained in a barrier facility on HEPA-filtered racks. The animals were fed with autoclaved laboratory rodent diet (Tekland LM-485; Western Research Products, Orange, CA). Animal experiments were performed in accordance with the National Research Council's "Guidelines for the Care and Use of Laboratory Animals" under NIH assurance number A3873-01.

Surgical Orthotopic Implantation (SOI) of MIA-PaCa-2-RFP Tumors. Red-fluorescent human pancreatic cancer xenografts were established in nude mice by SOI. Briefly, MIA-PaCa-2-RFP tumors in the exponential growth phase, grown *s.c.* in nude mice, were resected aseptically. Necrotic tissues were cut away, and the remaining healthy tumor tissues were cut with scissors and minced into 1-mm³ pieces in RPMI 1640. Each mouse was then anesthetized, and its abdomen was sterilized with alcohol. An incision was then created through the left upper abdominal pararectal line and peritoneum. The pancreas was carefully exposed, and two tumor pieces were transplanted onto the middle of the gland using a single 8-0 surgical suture (Davis-Geck, Inc., Manati, Puerto Rico). The pancreas was then returned into the peritoneal cavity, and the abdominal wall and the skin were closed in two layers using 6-0 surgical sutures. All procedures were performed with a $\times 7$ microscope (Olympus) or standard surgical loupes.

Surgical Resection of Primary Tumors. Each mouse in a treatment group requiring surgical resection of the primary pancreatic tumor was anesthetized, and sterilization of the abdomen was performed with alcohol. The peritoneum was subsequently reopened through the original surgical incision and an examination of adjacent structures was performed to ensure that macroscopic disease was localized to the pancreas. All grossly visible tumor was removed using sharp dissection. Hemostasis was achieved using 6-0 sutures. The abdomen was then closed in two layers as described previously.

Group Design and Treatment Schedule. Seven days after SOI, mice were randomized into eight groups of 10 mice each, depending upon whether they were to be treated by surgical resection of their primary tumor, chemotherapy, or both (Fig. 1). Mice in groups 1–4 were not treated surgically. Mice in group 1 did not receive chemotherapy and thus served as negative controls. Mice in groups 2–4 were treated with primary CS-682 (Sankyo Pharmaceuticals, Tokyo, Japan) at doses of 40, 50, or 60 mg/kg each treatment day, respectively, according to the treatment schedules outlined below.

Mice in groups 5–8 underwent surgical resection of their primary tumors 7 days after orthotopic implantation by a single blinded surgeon, as described previously. Mice in group 5 received no additional chemotherapy; mice in groups 6–8 received adjuvant CS-682 at doses of 40, 50, or 60 mg/kg each treatment day, respectively.

CS-682 was administered by oral gavage. Treatment with primary or adjuvant CS-682 was initiated 9 days after orthotopic tumor implantation (2 days after surgical resection when applicable) and was to be administered five times each treatment week for a total of 5 weeks or until death. As detailed below, mice in groups receiving 60 mg/kg did not tolerate chronic treatment and required a modification of the treatment schedule. In these groups, CS-682 was administered 9 times in weeks 1 and 2 after SOI and then 10 times in weeks 4 and 5, with a treatment hiatus during week 3 (Fig. 2).

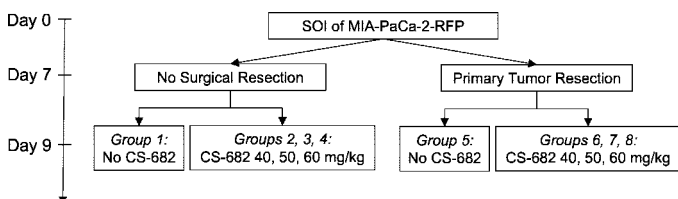


Fig. 1. Experimental protocol. Human MIA-PaCa-2-RFP pancreatic tumors were implanted orthotopically onto the pancreas of nude mice on day 0. On day 7, half of the mice underwent surgical resection of their primary tumor. Two days later, mice with unresected tumors were randomized to receive no treatment or primary CS-682 at oral doses of 40, 50, or 60 mg/kg, five times/week for 5 weeks or until death. Mice with resected tumors were randomized to receive no chemotherapy or adjuvant CS-682 at oral doses of 40, 50, or 60 mg/kg, five times/week for 5 weeks or until death.

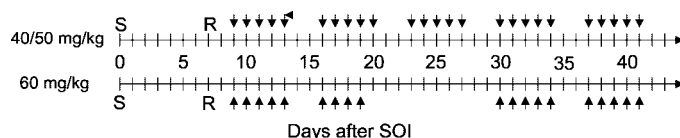


Fig. 2. Treatment schedule. According to the original treatment protocol, all groups receiving chemotherapy were to be treated five times/week for 5 weeks or until death. Mice in groups receiving a dose of 40 or 50 mg/kg were not significantly affected by toxicity, and therefore, the treatment schedule for these groups remained unaltered. In contrast, mice treated with a dose of 60 mg/kg were found to exhibit evidence of significant systemic toxicity after 9 doses. Chemotherapy administration was thus withheld until week 4, at which time, 10 additional doses were administered on schedule. S, surgical orthotopic implantation of tumors; R, primary tumor resection; \uparrow , \downarrow , CS-682 administration.

External *in Vivo* Whole-Body Imaging. Twice/week, mice were weighed and underwent external *in vivo* imaging. This was performed in a fluorescent light box illuminated by fiberoptic lighting at 470 nm (Lighttools Research, Encinitas, CA). Emitted fluorescence was collected through a long-pass filter GG475 (Chroma Technology, Battleboro, VT) on a Hamamatsu C5810 3-chip cooled color charge-coupled device camera (Hamamatsu Photonics Systems, Bridgewater, NJ). High-resolution images of 1024 \times 724 pixels were captured directly on an IBM PC or continuously through video output on a high-resolution Sony VCR model SLV-R1000 (Sony Corp., Tokyo, Japan). Images were processed for contrast and brightness and analyzed with the use of Image Pro Plus 3.1 software (Media Cybernetics, Silver Spring, MD). Real-time determination of tumor burden was performed by quantifying fluorescent surface area, as described previously (15, 16).

Direct Open Imaging and RFP Fluorescence Microscopy. Mice were sacrificed and explored when they appeared premonitory. After euthanasia, each mouse underwent laparotomy and sternotomy. Excitation of RFP in the light box, described above, facilitated identification of primary and metastatic disease by fluorescence visualization. After performing full-body open images, the solid organs were removed, and their surfaces were thoroughly examined for any evidence of metastases. Fluorescence microscopy was accomplished using a Leica fluorescence stereo microscope model LZ12 (Leica Microsystems, Inc., Bannockburn, IL) equipped with a mercury 50-W lamp power supply. Selective excitation of RFP was produced through a D425/60 band-pass filter and 470 DCXR dichroic mirror. Emitted fluorescence was collected by the Hamamatsu camera system described above.

Histological Analysis. Representative primary tumors and metastases were removed at the time of autopsy, fixed in 10% formalin, embedded in paraffin, and sectioned. Samples were subsequently processed with standard H&E staining for Brightfield microscopic examination.

Statistical Analysis. Differences among treatment groups were assessed using ANOVA and Student's *t* test using Statistica (Statsoft, Inc., Tulsa, OK). Kaplan-Meier analysis with a log-rank test was used to determine survival and differences between treatment groups. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Morphological and Growth Characteristics of MIA-PaCa-2-RFP *in Vitro*. RFP-expressing MIA-PaCa-2 cells appeared morphologically identical to their parent MIA-PaCa-2 cell line under light microscopy. We have previously demonstrated the growth rates of MIA-PaCa-2 and MIA-PaCa-2-RFP cells to be statistically equivalent (15). Primary and metastatic MIA-PaCa-2-RFP pancreatic tumors exhibited features of poorly differentiated pancreatic ductal adenocarcinoma on H&E staining (data not shown).

Analysis of Toxicity. The body weight and general appearance of each mouse were monitored and recorded twice weekly as evidence of systemic toxicity. The weights of mice that did not receive CS-682 either remained constant until death or rose gradually due to the accumulation of intra-abdominal ascites (Fig. 3). Wasting of body fat, most pronounced in the interscapular area of the back, was a common late finding that occurred in conjunction with disseminated disease.

At a dose of 40 mg/kg, treatment with CS-682 was not associated

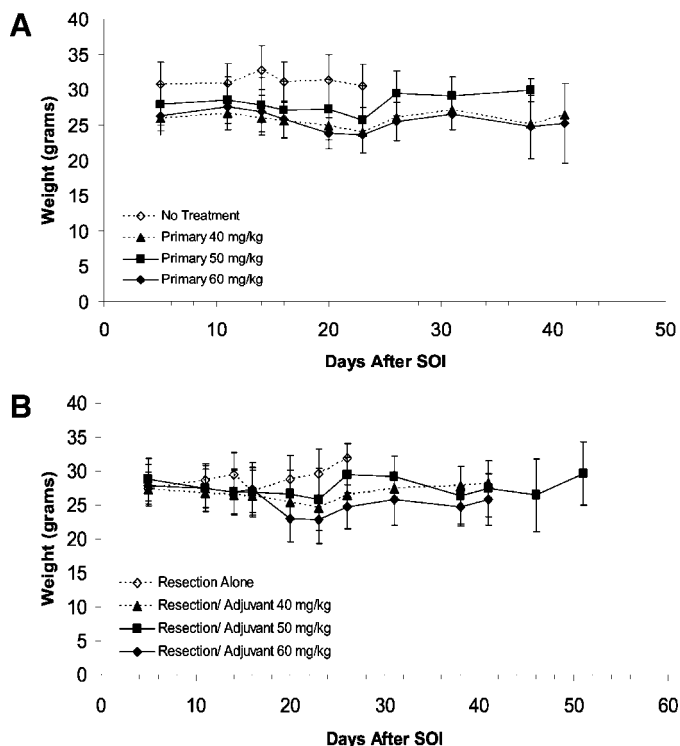


Fig. 3. Average body weight over the course of treatment for animals treated nonoperatively +/- primary CS-682 (A) and operatively +/- adjuvant CS-682 (B). Drug-associated weight loss was not significant for mice in groups treated with either 40 or 50 mg/kg using the schedules described in the "Materials and Methods." At an adjuvant dose of 60 mg/kg, average mouse body weight declined >15% from baseline by day 20 because of chronic drug toxicity, necessitating a temporary withdrawal of chemotherapy from both the primary and adjuvant 60 mg/kg groups. Values represent mean weight of animals in each treatment group alive at the given time point +/- SD.

with a significant loss in body weight. As in control groups, interscapular wasting of body fat was a late finding and was not observed in the absence of disseminated disease. Death in all mice, even those receiving long-term treatment, clearly occurred from disseminated pancreatic cancer, not drug toxicity.

In groups treated with 50 mg/kg CS-682, the effects of chronic drug administration were not sufficient to require a modification of the original treatment protocol. Nonetheless, a moderate decrease in body fat with interscapular wasting was frequently noted in the absence of disseminated pancreatic disease after 2 weeks of continuous CS-682 treatment, indicating a cumulative effect of drug administration over time. This effect was not severe and did not lead to a significant loss of body weight (Fig. 2). Even so, one mouse in each of the adjuvant and primary groups appeared to die from chronic drug toxicity, both after 2 complete weeks of chemotherapy.

Although no acute drug toxicity was observed in mice treated with 60 mg/kg CS-682, cumulative adverse effects were noted over the first 2 weeks of treatment in all mice, requiring termination of drug administration after the first nine doses (Fig. 2). By this time, interscapular fat wasting was noted to be severe in all animals, leading to a >15% loss in body weight by day 20. At this point, administration of CS-682 was aborted until week 4, allowing all animals to recover with concurrent gains in both body fat and weight, after which, treatment resumed for 2 more weeks. Using this strategy, only 1 animal was lost to toxicity on day 41. Death in all other animals did not occur in the absence of disseminated pancreatic disease.

Real-Time Analysis of Therapeutic Efficacy

In Vivo Characteristics of MIA-PaCa-2-RFP Tumor Growth. Real-time, fluorescence whole-body optical imaging revealed a progressive increase in locoregional and metastatic growth in all untreated animals after SOI of human MIA-PaCa-2-RFP pancreatic tumor fragments (Fig. 4). Fluorescent primary tumor was visible through the skin as early as 5 days after implantation and was visible in 70% of animals by day 10 and 100% of animals by day 14. The development of distant solid tumor metastases and intra-abdominal ascites were both early findings, identified in 60 and 100% of animals, respectively, by day 16. Noninvasive quantitative measurements of externally visible fluorescent area enabled the construction of *in vivo* tumor growth curves, which demonstrated a remarkably linear tumor growth rate in the untreated animals that led to death from disease in all mice by 30 days and a median survival of 26 days (Table 1). Upon

Fig. 4. Sequential whole-body images of 1 representative mouse from each of four treatment groups. Real-time evaluation of therapeutic interventions on pancreatic tumor growth and metastasis was facilitated in this noninvasive system using fluorescence visualization. Resection was performed on day 7 in both the resection and adjuvant groups. CS-682 was initiated in the primary and adjuvant groups at a dose of 50 mg/kg on day 9 as detailed in "Materials and Methods." The inhibitory effects of each treatment group on the growth and dissemination of orthotopically implanted human pancreatic tumors were visible in the live mouse by RFP fluorescence.

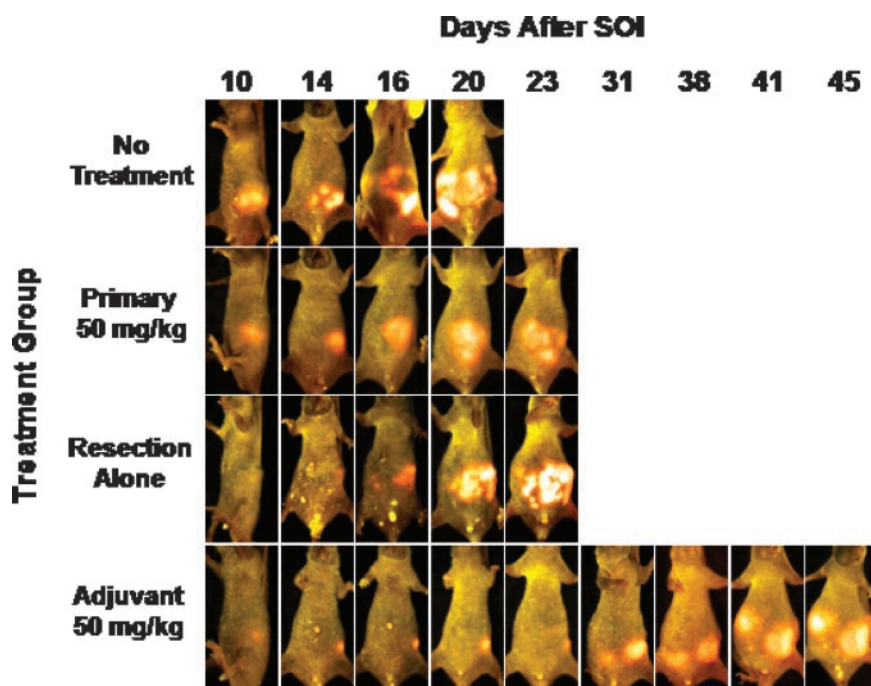


Table 1 Survival data for all eight treatment groups

Both primary and adjuvant CS-682, at all doses, significantly prolonged survival compared to no treatment and was more effective than surgery alone at doses of 50 and 60 mg/kg. *P*s represent log-rank test of given treatment group versus no treatment, resection only, and same dose of CS-682, respectively. NT, no treatment.

	Median survival	25% survival	<i>P</i> versus NT	<i>P</i> versus resection	<i>P</i> versus drug alone
No treatment	26	26		0.03	–
CS 682 40 mg/kg	31	38.5	0.03	0.13	–
CS 682 50 mg/kg	34	37.5	0.01	0.045	–
CS 682 60 mg/kg	38.5	50.5	0.009	0.03	–
Resection alone	28	30	0.03	–	–
Resection + CS40	39	41	0.03	0.09	0.25
Resection + CS50	48	57.5	0.002	0.004	0.03
Resection + CS60	40	54.5	0.02	0.03	0.39

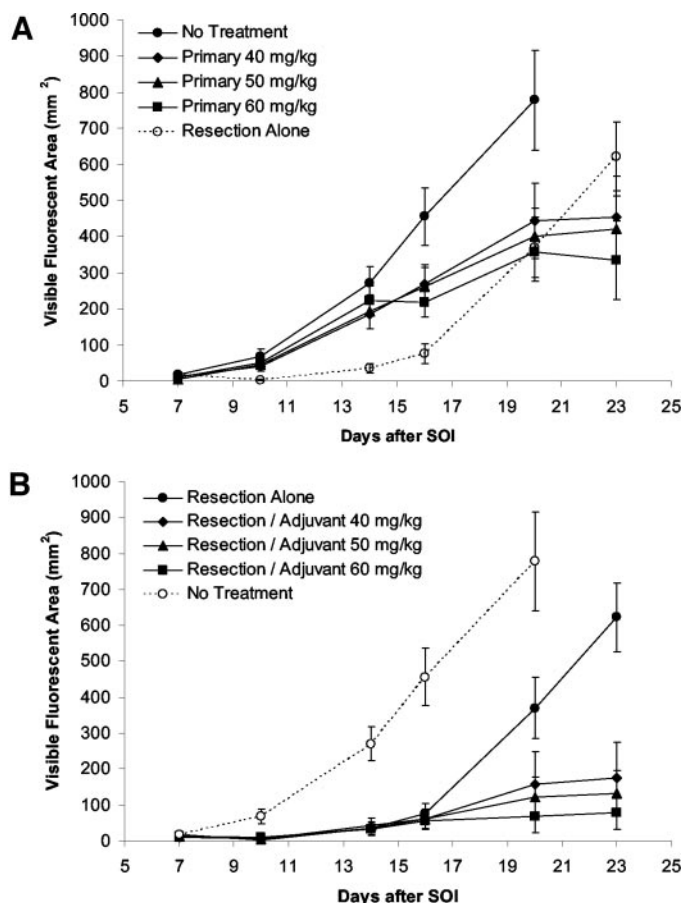


Fig. 5. Quantification and real-time analysis of pancreatic tumor growth in the early stages of development *in vivo* demonstrated the mechanisms whereby survival was enhanced by either primary (A) or adjuvant (B) CS-682. Tumor growth in surgically treated mice was characterized by a transient reduction in tumor load after resection, after which, tumor growth occurred at the same rate as in untreated animals. CS-682 exerted its effects by retarding the rate of tumor growth. Combining surgical tumor reduction with CS-682-induced growth suppression led to a synergistic effect on early tumor growth that corresponded with a dramatic increase in survival.

autopsy, metastases were confirmed in multiple sites, including the diaphragm, intestinal and portal lymphatics, retroperitoneum, kidney, and liver.

Surgical Resection. Early surgical resection of primary disease led to a marginal yet significant increase in survival (median survival, 28 days, $P = 0.03$). In all cases, resection was performed before accumulation of any distant metastatic deposits and entailed removal of all gross pancreatic disease, with a concurrent, significant reduction in fluorescent tumor area visible externally by day 10 ($P = 0.009$, Fig. 5). Nonetheless, the benefits of surgery were clearly transient. Recurrent tumor burden increased progressively after day 10, reaching preoperative levels by day 14. At this point, tumor enlargement and

dissemination accelerated, with an average tumor growth rate similar to that seen in the no treatment group. As expected, surgical resection also postponed the development of ascites, with only 20% of animals exhibiting this clinical finding on day 16.

Primary CS-682 Treatment. In concordance with our previous results (13), primary administration of CS-682 at each dose tested significantly enhanced the survival of mice with orthotopically implanted MIA-PaCa-2-RFP tumors ($P < 0.05$ for each dose, Table 1). The survival advantage conferred by CS-682 was similar using each dose tested ($P = 0.4$). At doses of 50 and 60 mg/kg, a significant increase in overall survival was also achieved over surgical resection alone ($P = 0.045$ and 0.03, respectively) by the primary administration of CS-682.

Real-time whole-body imaging of early tumor growth (Fig. 5) confirmed that the favorable effect of CS-682 chemotherapy on survival was caused by a significant reduction in the rate of tumor growth by this agent. Although mice treated primarily with CS-682 had more tumor than those treated surgically over the first 2–3 weeks after implantation, the growth-suppressive effects of CS-682 outlasted the transient effects of surgical tumor resection, with an intersection in the growth curves at 21 days.

Resection and Adjuvant CS-682 Treatment. The largest increase in survival was achieved by the postoperative administration of CS-682 after surgical resection. At all doses tested, mice treated in this manner had a significant increase in survival over treatment controls ($P < 0.05$). On the 50 and 60 mg/kg regimens, the enhancement in survival was also significant compared with resection alone ($P < 0.004$ and 0.03, respectively). Enhancement of survival was most significant at a dose of 50 mg/kg. On this regimen, mice had a median survival of 48 days and 30% lived at least 60 days (Fig. 6). Using fluorescence visualization, the growth-suppressive effects of this combination therapy was evident: on day 23, after 40% of untreated animals had already succumbed to disseminated disease,

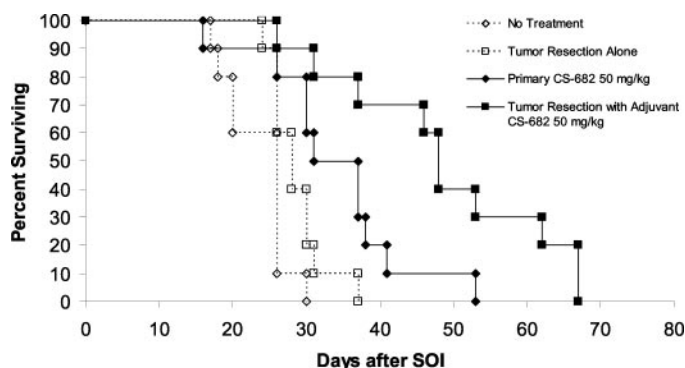


Fig. 6. Resection of orthotopically implanted MIA-PaCa-2-RFP human pancreatic tumors and postoperative treatment with oral CS-682 at a dose of 50 mg/kg led to a highly significant survival advantage over either no treatment ($P = 0.002$) or surgical resection alone ($P = 0.004$); median survival, 48, 26, and 28 days, respectively. Treated with CS-682 at 50 mg/kg and surgical resection, 30% of animals survived >60 days.

70% of animals in the adjuvant 50 mg/kg group had, at most, local disease confined to one abdominal quadrant. In the 30% of mice that experienced particularly long-term survival (>60 days) on this regimen, distant metastasis was not seen until ~10 days after the completion of chemotherapy, at which time, the development of metastasis accelerated, and the mice ultimately succumbed to disseminated pancreatic disease. Construction of *in vivo* tumor growth curves demonstrated apparent synergism between surgical resection and the application of adjuvant CS-682 in the suppression of tumor growth (Fig. 5). As expected, the development of ascites was also suppressed by adjuvant therapy, with only 20% of animals showing signs of fluid retention at the time of their death.

DISCUSSION

The benefits of adjuvant chemotherapy in the treatment of several solid malignancies are well described. Using adjuvant strategies, cytotoxic agents or other therapies are administered after surgical resection of all gross tumor in an attempt to destroy microscopic disease left behind that might otherwise lead to tumor recurrence. Delivered postoperatively, certain chemotherapeutics can theoretically exert their greatest effects, with high drug doses, early administration times, and small volumes of residual disease being important principles of adjuvant therapy (17).

Although the use of adjuvant chemotherapy is routine in patients with other surgically treated malignancies, its role in the treatment of patients with pancreatic ductal adenocarcinoma is unclear. Presently, the only potentially curative treatment for such patients is complete surgical resection of tumor by pancreaticoduodenectomy or distal pancreatectomy. Over the past two decades, the 30-day mortality associated with these procedures has fallen to <4% at major centers (4, 18). Nonetheless, this improvement in immediate surgical outcomes has not increased median survival of patients with resected disease over 2 years because of unacceptably high rates of primary treatment failure and tumor recurrence.

Results of an early adjuvant trial in 1985 (19), which demonstrated a clear benefit to postoperative chemoradiation with 5-fluorouracil, led to optimism that tumor recurrence could be minimized and survival could be prolonged through the use of adjuvant therapies. Unfortunately, the data accumulated since then have not been uniformly supportive of these findings (11, 12, 20, 21). Almost 20 years later, the role of adjuvant therapies in pancreatic cancer is controversial, with most patients receiving adjuvant therapy only as part of one of many ongoing clinical trials. Nonetheless, the progress made in minimizing perioperative morbidity has opened the door to adjuvant therapy for an increasing number of patients. New, well-tolerated, highly effective adjuvant therapeutics would therefore be a welcome addition to the multimodality treatment of patients with this aggressive disease.

In this study, we demonstrate the ability of the novel agent CS-682 to delay recurrence and prolong survival in a highly aggressive mouse model of pancreatic cancer. This 2'-deoxycytidine analogue (22) has been shown to inhibit tumor growth by both inhibiting DNA polymerase and by inducing DNA self-strand breakage through incorporation of an active metabolite into the strands (23). Oral CS-682 has been shown to possess potent cytotoxic activity against several tumor cell lines *in vitro* and *in vivo* (23) and has been demonstrated to inhibit the development of liver metastasis (24). We have reported the efficacy of this agent in inhibiting growth and dissemination of pancreatic cancer in an orthotopic model and demonstrated that this ability may be caused by a direct inhibitory effect on the development of metastases (13).

The results of the present study clearly indicate that a survival

benefit can be achieved by administering CS-682 in the postoperative period after resection of all clinically evident macroscopic disease. This survival benefit is more significant than that which can be achieved by either surgery or primary CS-682 therapy alone. Using our noninvasive fluorescence imaging system, we were able to visualize the effects of each of these therapeutic modalities on a real-time basis. By physically removing the tumor, surgical resection decreased the amount of pancreatic tumor load compared with untreated animals in the early postoperative period. Unfortunately, the effects of this intervention were transient, and once sufficient residual disease was established, tumor growth and dissemination proceeded at a rate similar to that in controls. In contrast, primary CS-682 administration reduced the rate of pancreatic tumor growth, the subsequent beneficial effects of which were longer lasting than those of surgical manipulation. Delivering CS-682 postoperatively after tumor resection had the most favorable effect on survival.

The issue of chemotherapeutic toxicity of particular importance in designing adjuvant treatment protocols in which a necessity for high-dose intensity (17) must be balanced with an obvious need for a maintenance of quality of life in a population of patients that has already undergone major surgery (25). In this study, we have shown that a dose of CS-682 of 40 mg/kg is well tolerated, even when administered five times/week for a prolonged length of time. A dose of 50 mg/kg was also well tolerated by the majority of animals, although clinical effects were observed after daily chronic dosing. Pauses in treatment, necessitated in the 60 mg/kg groups, could temporize the effects seen using a daily treatment schedule.

In summary, we have demonstrated that the adjuvant use of CS-682 may be used after primary tumor resection to delay recurrence and prolong survival in our clinically relevant animal model of pancreatic cancer. Additional studies will be necessary to define a precise adjuvant treatment protocol in combination therapy with other cytotoxic agents for a curative strategy.

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