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ORIGINAL ARTICLE

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Enhanced antitumor activity of irofulven in combination with irinotecan in pediatric solid tumor xenograft models

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Abstract *Purpose*: Irofulven, a novel chemotherapeutic agent with a broad spectrum of activity, is effective against preclinical models of pediatric tumors. The cytotoxic activity of irofulven is augmented when combined with agents that interact with DNA topoisomerase I; however, none of the reported studies have used the protracted dosing schedule found to be active clinically in treatment of childhood cancers. The objective of this study was to evaluate the antitumor activity of irofulven in combination with irinotecan administered on a protracted schedule in a panel of pediatric solid tumor xenografts. *Methods*: Irofulven and irinotecan were evaluated alone or in combination against eight independent xenografts, which included childhood brain tumors ($n=5$), neuroblastoma ($n=1$), and rhabdomyosarcoma ($n=2$). Irofulven was administered i.v. daily for 5 days with courses repeated every 21 days for a total of three cycles. Doses of irofulven ranged from 1.33 to 4.6 mg/kg. Irinotecan was given i.v. daily for 5 days each week for 2 weeks repeated every 21 days for three cycles at doses between 0.28 and 1.25 mg/kg. *Results*: Irofulven and irinotecan, given as single agents, induced few responses in pediatric solid tumor xenografts at the selected doses. At the same doses, irofulven in combination with irinotecan demonstrated superior antitumor activity, inducing complete responses in seven of the eight xenograft lines. *Conclusions*: These studies show that the cytotoxic activity of irofulven is greater when combined with protracted administration of irinotecan. Although the systemic exposure of irofulven required to induce objective responses in this panel of pediatric solid tumors was in excess of that achievable in patients receiving maximally tolerated doses using this schedule of drug administration, the enhanced activity of irofulven in combination with irinotecan supports the pursuit of alternative administration strategies and combinations.

Keywords Irofulven · Irinotecan · Pediatrics · Solid tumors · Xenografts

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Introduction

Irofulven is a semisynthetic analog of the sesquiterpene toxin illudin S, a natural product of the *Omphalotus* mushroom [24]. It is a potent cytotoxic agent with a chemical structure different from chemotherapeutic agents currently in clinical use. It possesses a wider therapeutic index compared to its parent compound and it is preferentially cytotoxic to tumor cells in vitro [14, 16, 23, 25, 26, 31]. Irofulven rapidly enters cells and induces a unique form of DNA damage, although the specific lesion has not been identified. Irofulven has demonstrated efficacy in a variety of solid tumor xenograft models including those that express MDR1 or MRP [14, 15, 17, 23]. Irofulven inhibits DNA synthesis with cell cycle arrest in S phase [32], but does not produce interstrand DNA crosslinks or DNA-protein crosslinks. Irofulven retains its cytotoxic activity against tumor cells regardless of p53 or p21 status [12]. It is in various phases of clinical evaluation against many solid tumors.

Although results of preclinical studies have been encouraging, treatment with irofulven as a single agent is unlikely to be curative and the systemic exposure required for objective responses is in excess of that achievable in patients at tolerable doses [21]. Hence,

there is clinical interest in combining irofulven with other antineoplastic agents. Combinations with established chemotherapeutic agents have been studied both in vitro and in vivo and have demonstrated additive and/or synergistic antitumor activity, but there is no agreement as to the most effective combinations. Simultaneous combinations of irofulven and vinca alkaloids (vinblastine, vincristine, or vinorelbine), paclitaxel [19], cisplatin [18, 27], or 5-fluorouracil [27] typically demonstrated at least additive cytotoxicity in vitro. However, there are schedule-dependent differences. When vincristine or vinorelbine is added after irofulven, the interaction is antagonistic [19]. When docetaxel is added simultaneously or before irofulven, the interaction is synergistic, but antagonistic if docetaxel is added after irofulven [19]. When cisplatin is followed by irofulven the interaction is additive, but when cisplatin follows irofulven the interaction is antagonistic [27]. Synergy has also been reported for irofulven combined with aziridine-containing drugs, such as thiotepa and mitomycin C [20]. Some results of xenograft studies have been consistent with those from cell culture, as greater than anticipated antitumor activity was observed for combinations of irofulven with thiotepa or mitomycin C [20].

The combination of irofulven with agents that target DNA topoisomerase I has demonstrated in vitro and in vivo synergy. Irofulven has been shown to enhance the cytotoxicity of topotecan against rhabdomyosarcoma, promyelocytic leukemia, and non-small-cell lung cancer xenografts [6, 18, 30], and to enhance the activity of irinotecan against colon carcinoma xenografts [1]. Of interest to us, irinotecan has been shown to be active against xenografts derived from pediatric tumors, including neuroblastomas [29], brain tumors [11], and rhabdomyosarcomas [10].

Irinotecan, a camptothecin prodrug that is activated by carboxylesterases to the active metabolite SN-38, converts its target DNA topoisomerase I into a cellular poison. SN-38 stabilizes the covalent complex between DNA topoisomerase I and cleaved DNA, which shifts the equilibrium toward the enzyme-bound cleavable complex. This increases the probability of a collision with a replication fork, and thus generates a double-strand DNA break, or stalling of the replication fork during S phase, leading to a cascade of events that result in cell death. Both preclinical xenograft and clinical studies have shown that the antitumor activity of irinotecan is highly schedule-dependent. Protracted administration schedules are more effective than more intense schedules of shorter duration when similar total dosages are given [29].

The interest in developing a combination regimen such as irofulven and irinotecan stems not only from the initial results that this combination augments cytotoxic activity, but also from the fact hat these agents have relatively nonoverlapping toxicities, where the limiting clinical toxicity of irofulven is myelosuppression and that of irinotecan is diarrhea. Nevertheless, xenograft studies of irofulven have generally employed doses that produce a systemic exposure in excess of that achievable in patients, and when used in combination with DNA topoisomerase I poisons, the more active protracted dosing schedule was not utilized. By using a protracted dosing schedule for irinotecan, the dose of irofulven may be decreased such that the systemic exposure in xenografts may be achieved in patients without adversely affecting antitumor activity. In this study, we investigated the activity of the combination of irofulven and irinotecan given on a protracted schedule against a panel of childhood solid tumor xenografts and determined whether the response to the combination was greater than that expected from the response to either drug alone.

Materials and methods

SCID mice

CB17/Icr *scid*-/- female mice were implanted with tumor fragments of about 3 mm^3 in size in the space of the dorsal lateral flank of each mouse to initiate tumor growth. Tumor-bearing mice were randomized into groups of six or seven before initiating therapy. All mice were maintained under barrier conditions. All experiments were performed using protocols and conditions approved by the Institutional Animal Care and Use Committee.

Tumor lines

In this study, the antitumor activities of irofulven and irinotecan were examined individually and in combination with the following eight pediatric solid tumor lines which have been characterized and previously described [2, 4, 7, 9, 11, 21, 29]: SJ-GBM2 (glioblastoma), SJ-BT27 (primitive neuroectodermal tumor), SJ-BT29 (atypical teratoid rhabdoid tumor), SJ-BT33 (giant cell astrocytoma), DAOY (medulloblastoma), NB-1771 (neuroblastoma), and Rh18 and Rh30 (rhabdomyosarcoma). All of the experiments were conducted in tumors within 30 serial passages of engraftment. Transplantations were from mouse to mouse.

Growth inhibition studies

Each mouse bearing subcutaneous tumors received the chemotherapeutic agent(s) when the tumors were about 0.20–1 cm in diameter, as reported previously [11]. Briefly, two perpendicular tumor diameters were measured at 7-day intervals with digital vernier calipers interfaced with a Macintosh computer. Assuming tumors to be spherical, volumes were calculated from the formula $(\pi/6)\times d^3$, where d represents the mean

diameter. Tumor volumes were determined up to 12 weeks after starting treatment.

Drug formulation and administration

Irofulven was provided by MGI Pharma, through the Cancer Treatment Evaluation Program, National Cancer Institute, or directly from MGI Pharma (Bloomington, Minn.). The drug (10 mg) was dissolved in 0.1 ml absolute ethanol and after 5 min, 9.9 ml 5% dextrose in water (D5W) was added. Irofulven was administered i.v. daily for 5 days repeated every 21 days for three cycles [(d×5)1]3. Irinotecan was obtained commercially from Pharmacia (Kalamazoo, Mich.) and was diluted in D5W. It was given i.v. daily for 5 days for two consecutive weeks with cycles repeated every 21 days for three cycles [(d×5)2]3.

Tumor response and tumor failure time

For individual tumors, partial response (PR) was defined as a volume regression of $>50\%$, but with measurable tumor (≥ 0.10 cm^3) at all times. Complete response (CR) was defined as disappearance of measurable tumor mass (<0.10 cm^3) at any point within 12 weeks after initiation of therapy. Maintained complete response (MCR) was CR without tumor regrowth within the 12-week study period. If the initial tumor volume was <0.20 cm^3, data on that tumor were excluded.

Tumor failure time was defined as the time (in weeks) required by individual tumors to quadruple their volume from the initiation of therapy. Tumor failure times were termed as censored if a mouse died prior to week 12 and before a tumor grew to four times its initial volume.

Statistical methods

For comparisons of time to tumor failure for different treatment regimens, survival distributions of each treatment group were compared to the survival distribution of the control group using the exact log-rank test. Experiment-wise significance levels were maintained at 0.05 using the Bonferroni procedure [8] to adjust for the multiplicity of tests of significance within each tumor line/study. SAS version 8.2 (with Proc StatXact) was used for statistical analysis. Direct comparisons of tumor failure times between combination studies and their respective single-agent studies were performed using the exact log-rank test.

Results

Drug dose and scheduling

The sensitivities of the pediatric tumors used in this study to irofulven or irinotecan individually have been previously reported [11, 21]. As a single agent, the maximum tolerated dose of irofulven is 4.6 mg/kg, but the resultant systemic exposure is about 15-fold in excess of the recommended phase II dosage for adult patients with solid tumors [21]. As a result, irofulven doses predominantly below the MTD were utilized in the combination studies, ranging from 1.3 to 4.6 mg/kg daily for 5 days repeated every 21 days for three cycles. Irinotecan was administered at dose levels between 0.28 and 1.25 mg/kg daily for 5 days on two consecutive weeks repeated every 21 days for three cycles, which give plasma SN-38 lactone systemic exposures consistent with those achieved in patients receiving irinotecan using the same schedule of administration [5, 29]. A daily systemic exposure to SN-38 lactone of 99 and 257 ng h/ml was associated with PRs and CRs, respectively, in neuroblastoma xenografts [29]. The SN-38 lactone AUC from the upper dose of 1.25 mg/kg used in the combination studies would be about 115 ng h/ml. Dose levels of irofulven and irinotecan were chosen so that neither drug alone caused CRs; dose levels for irofulven were $<70\%$ of the MTD (in all but one xenograft) and for irinotecan were $<15\%$ of the MTD. These studies focused on antitumor activity, rather than on mouse toxicity.

Control of tumor growth

Irofulven or irinotecan alone and in combination were evaluated against one neuroblastoma, two rhabdomyosarcoma, and five brain tumor xenografts. The statistical analysis of the antitumor activity of single agents and combination treatments is summarized in Table 1. Results of the exact log-rank tests used to compare differences in the distributions of tumor failure times (i.e., time for tumors to grow to four times their volume at the start of treatment) among various treatment combinations and respective control groups are presented. The exact log-rank test was used to compare each treatment group with the control group and also to compare the combination treatments with individual agents at the same dose levels. The P values for these tests were adjusted by the Bonferroni procedure [8], where the P value of a test is multiplied by the number of tests. Table 1 also shows the number of mice that achieved PR and CR. In all tumor lines, at least one of the treatment groups showed significant improvement in tumor growth control over the control groups.

The activity of combinations was significantly better than the activity of either agent used at the same dose level, with a few exceptions. For example, in studies where single-agent therapy induced significant rates of response (e.g., Rh30), it was not possible to determine whether combinations were superior to monotherapy. All treatment groups in this line showed improvement over the control group. None of the mice receiving the combination of irofulven and irinotecan or irofulven alone had tumors that grew to four times the initial volume

Table 1 Comparison of the effect of individual and combination treatments by time to tumor failure

Treatment	Group	Time to 4× initial tumor volume ± SD (weeks)	Adjusted *P* value[a]	Number of PRs	Number of CRs	Number of MCRs[b]
SJ-GBM2						
Control	A	2.7 ± 0.2	–	0	0	0
Irofulven 3.0 mg/kg [(d×5)1]3 i.v.	B	4.4 ± 0.4	0.514	0	0	0
Irofulven 3.0 mg/kg [(d×5)1]3 i.v. + irinotecan 1.25 mg/kg [(d×5)2]3 i.v.	C	> 12	0.042	0	7	7
Irinotecan 1.25 mg/kg [(d×5)2]3 i.v.	D	5.3 ± 0.2	0.031	0	0	0
Irofulven 3.0 mg/kg [(d×5)1]3 i.v. + irinotecan 0.61 mg/kg [(d×5)2]3 i.v.	E	> 12	0.042	0	7	6
Irinotecan 0.61 mg/kg [(d×5)2]3 i.v.	F	5.6 ± 0.3	0.047	0	0	0
	C vs B		0.005			
	E vs B		0.005			
	C vs D		0.005			
	E vs F		0.005			
SJ-BT27						
Control	A	2.4 ± 0.2	–	0	0	0
Irofulven 2.0 mg/kg [(d×5)1]3 i.v.	B	6.8 ± 2.2	0.052	2	1	1
Irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	C	11.0 ± 0.2	0.005	4	0	0
Irofulven 2.0 mg/kg [(d×5)1]3 i.v. + irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	D	> 12	0.005	0	7	7
Irofulven 1.33 mg/kg [(d×5)1]3 i.v.	E	7.5 ± 1.0	0.021	0	0	0
Irofulven 1.33 mg/kg [(d×5)1]3 i.v. + irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	F	> 12	0.005	0	6	6
	D vs B		0.629			
	F vs E		0.020			
	D vs C		0.005			
	F vs C		0.042			
SJ-BT29						
Control	A	2.7 ± 0.2	–	0	0	0
Irofulven 3.0 mg/kg [(d×5)1]3 i.v.	B	9.5 ± 12	0.016	0	0	0
Irinotecan 1.25 mg/kg [(d×5)2]3 i.v.	C	9.0 ± 3.0	0.016	0	0	0
Irofulven 3.0 mg/kg [(d×5)1]3 i.v. + irinotecan 1.25 mg/kg [(d×5)2]3 i.v.	D	> 12	0.005	1	6	4
Irinotecan 0.61 mg/kg [(d×5)2]3 i.v.	E	9.6 ± 0.8	0.016	0	0	0
Irofulven 3.0 mg/kg [(d×5)1]3 i.v. + irinotecan 0.61 mg/kg [(d×5)2]3 i.v.	F	> 12	0.005	2	5	4
	D vs B		0.629			
	F vs B		0.629			
	D vs C		1.000			
	F vs E		0.042			
SJ-BT33						
Control	A	2.7 ± 0.3	–	0	0	0
Irofulven 4.6 mg/kg [(d×5)1]3 i.v.	B	3.0 ± 0.0	1.000	0	0	0
Irinotecan 1.25 mg/kg [(d×5)2]3 i.v.	C	3.0 ± 0.0	1.000	0	0	0
Irofulven 4.6 mg/kg [(d×5)1]3 i.v. + irinotecan 1.25 mg/kg [(d×5)2]3 i.v.	D	2.0	1.000	0	0	0
Irinotecan 0.61 mg/kg [(d×5)2]3 i.v.	E	3.0 ± 0.0	1.000	0	0	0
Irofulven 4.6 mg/kg [(d×5)1]3 i.v. + irinotecan 0.61 mg/kg [(d×5)2]3 i.v.	F	2.0 ± 0.0	0.042	0	0	0
	D vs B		0.005			
	F vs B		0.005			
	D vs C		0.147			
	F vs E		0.005			
NB-1771						
Control	A	2.9 ± 0.1	–	0	0	0
Irofulven 2.0 mg/kg [(d×5)1]3 i.v.	B	3.4 ± 0.2	1.000	0	0	0
Irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	C	3.7 ± 0.2	0.163	0	0	0
Irofulven 2.0 mg/kg [(d×5)1]3 i.v. + irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	D	> 12	0.006	0	7	7
Irofulven 1.33 mg/kg [(d×5)1]3 i.v.	E	3.0 ± 0.0	1.000	0	0	0
Irofulven 1.33 mg/kg [(d×5)1]3 i.v. + irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	F	12.0 ± 0.0	0.006	1	6	1
Irofulven 3.0 mg/kg [(d×5)1]3 i.v.	G	8.3 ± 0.6	0.006	0	0	0
	D vs B		0.006			
	F vs E		0.006			
	D vs C		0.006			
	F vs C		0.006			

Table 1 (Contd.)

Rh18						
Control	A	1.7 ± 0.2	–	0	0	0
Irofulven 2.0 mg/kg [(d×5)1]3 i.v.	B	4.2 ± 1.0	0.005	2	0	0
Irofulven 1.33 mg/kg [(d×5)1]3 i.v.	C	3.0 ± 0.0	0.005	0	2	2
Irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	D	11.6 ± 0.4	0.005	3	0	0
Irofulven 2.0 mg/kg [(d×5)1]3 i.v. + irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	E	> 12	0.005	0	7	7
Irofulven 1.33 mg/kg [(d×5)1]3 i.v. + irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	F	> 12	0.005	1	6	6
	E vs B		0.189			
	F vs C		0.189			
	E vs D		0.005			
	F vs D		1.000			
Rh30						
Control	A	2.6 ± 0.2	–	0	0	0
Irofulven 2.0 mg/kg [(d×5)1]3 i.v.	B	> 12	0.005	0	7	7
Irofulven 1.33 mg/kg [(d×5)1]3 i.v.	C	> 12	0.005	0	7	7
Irinotecan 0.28 mg/kg [(d×5)2]3 i.v.	D	11.7 ± 0.2	0.005	7	0	0
Irofulven 2.0 mg/kg [(d×5)1]3 i.v. + irinotecan 0.28 mg/kg [(d×5)2]3 i.v.	E	> 12	0.005	0	7	7
Irofulven 1.33 mg/kg [(d×5)1]3 i.v. + irinotecan 0.28 mg/kg [(d×5)2]3 i.v.	F	> 12	0.005	0	7	7
	E vs B		1.000[c]			
	F vs C		1.000[c]			
	E vs D		0.005			
	F vs D		0.005			
DAOY						
Control	A	2.6 ± 0.2	–	0	0	0
Irofulven 1.33 mg/kg [(d×5)1]3 i.v.	B	3.0 ± 0.0	1.000	0	0	0
Irinotecan 0.28 mg/kg [(d×5)2]3 i.v.	C	12.0	0.005	1	6	1
Irofulven 1.33 mg/kg [(d×5)1]3 i.v. + irinotecan 0.28 mg/kg [(d×5)2]3 i.v.	D	> 12	0.005	0	7	7
Irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	E	> 12	0.005	0	7	0
Irofulven 1.33 mg/kg [(d×5)1]3 i.v. + irinotecan 0.4 mg/kg [(d×5)2]3 i.v.	F	> 12	0.005	0	7	6
	D vs B		0.005			
	F vs B		0.005			
	D vs C		1.000			
	F vs E		1.000[c]			

[a] *P* values obtained using the Bonferroni correction procedure.
[b] Fraction of CRs maintained through week 12.
[c] *P* values obtained from asymptotic results; exact log-rank test was unavailable since there were no events (i.e., treatments were equivalent)

($P = 1$ when compared to irofulven alone; Table 1), even though the combination brought about CRs more rapidly than monotherapy. On the other end of the spectrum, single-agent and combination therapy had no antitumor activity against SJ-BT33 subependymal giant cell astrocytoma xenografts. Despite differences in tumor failure times between the combination and single agents, there were no PRs or CRs observed and all lines rapidly quadrupled their initial tumor volumes.

In the other six tumor lines, irofulven combined with irinotecan demonstrated superior activity against tumors at dose levels that had little activity when administered as single agents. In the remaining three brain tumor xenografts the combination showed improved tumor control over individual treatments. Figure 1 shows the dramatic effects of combination treatment against the glioblastoma line SJ-GBM2. None of the mice in the combination treatment groups had tumors that quadrupled in volume, and every mouse had a CR to treatment, 13 (93%) of which were maintained at 12 weeks. In contrast, all tumors treated with single agents grew to four times their initial volume. Similarly, the combination treatment induced CRs in 100% of SJ-BT27 PNET xenografts that were maintained at week 12 (Fig. 2). Irofulven and irinotecan as single agents had marginal activity, with the majority of tumors increasing in size to four times their initial volume. Irofulven monotherapy also exhibited a steep dose–response effect. The higher dose of 2 mg/kg produced two PRs (29%) and one CR (14%), but dose de-escalation to 1.33 mg/kg resulted in no responses. Irinotecan alone induced four PRs (57%) and no CRs. Against SJ-BT29 ATRT xenografts, the combination of irofulven 3.0 mg/kg and irinotecan 1.25 mg/kg induced one PR (14%) and six CRs (86%), four (57%) of which were maintained at week 12. Despite a decrease in the dose of irinotecan to 0.61 mg/kg in the combination regimen, response rates remained similar: 29% PR, 71% CR, and 57% CR maintained at week 12. None of the tumors quadrupled in volume following combination treatment.

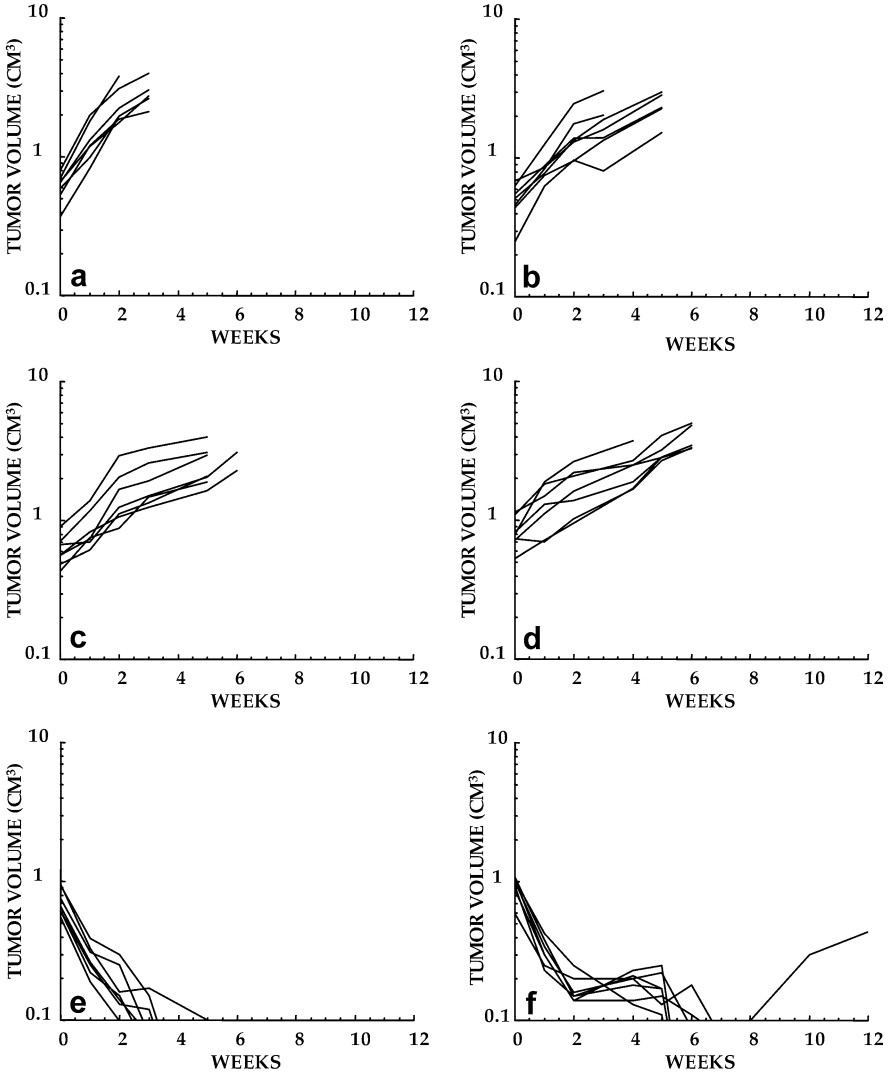

Fig. 1 Responses of SJ-GBM2 glioblastoma xenografts to single-agent irofulven, irinotecan or binary drug combinations. **a** Control; **b** irofulven 3.0 mg/kg; **c** irinotecan 1.25 mg/kg; **d** irinotecan 0.61 mg/kg; **e** irofulven 3.0 mg/kg + irinotecan 1.25 mg/kg; **f** irofulven 3.0 mg/kg + irinotecan 0.61 mg/kg. Irofulven was administered intravenously daily for 5 days every 21 days for three cycles, designated [(d×5)1]3. Irinotecan was administered intravenously daily for 5 days on two consecutive weeks repeated every 21 days for three cycles, designated [(d×5)2]3. Irofulven was administered during the first 5 days of irinotecan treatment in each cycle

Although there was little difference in tumor failure times between combination and single-agent treatments ($P = 0.629$ against irofulven alone and $P = 1$ against irinotecan 1.25 mg/kg alone), there were no responses seen with monotherapy (Table 1).

In the NB-1771 neuroblastoma line, significant improvement in time to tumor failure was noted with the use of combination treatment ($P = 0.006$ compared to irofulven or irinotecan alone; Table 1). When irofulven 2.0 mg/kg and irinotecan 0.4 mg/kg were administered concurrently, every mouse had a CR. These CRs were maintained at week 12 (Fig. 3). When the irofulven dose was decreased to 1.33 mg/kg in the combination regimen, the response rates were 14% PR and 86% CR, although there was tumor regrowth in all but one mouse. Irofulven and irinotecan had little activity as single agents against NB-1771, with no tumor regression or growth inhibition to quadruple initial tumor volumes (Fig. 3).

Against Rh18 rhabdomyosarcoma xenografts, combination treatment showed only marginally superior activity over single agents, but there was a considerable biological gain in the combination treatment groups (Table 1). Combination treatment induced seven CRs at an irofulven dose of 2.0 mg/kg and six CRs and one PR at a dose of 1.33 mg/kg. There was no tumor regrowth in any combination group over the 12-week observation period. Corresponding single-agent irofulven treatment doses produced two PRs and two CRs, respectively, and irinotecan monotherapy resulted in three PRs. The remaining tumors grew to four times their initial volumes.

Against DAOY medulloblastoma lines, the combination treatment showed significant differences over irofulven ($P = 0.005$) but not over irinotecan ($P = 1$; Table 1). Irinotecan alone seemed to control tumor growth quite well, although the antitumor effects were not as long-lasting as those seen after combination treatment. None of the mice in the drug combination groups had tumors that quadrupled in volume; every mouse had a CR to treatment, and all but one CR was maintained at 12 weeks (93%). In contrast, there were a greater proportion of tumor regrowths with mice treated with irinotecan as a single agent, as only 14% and 0% of

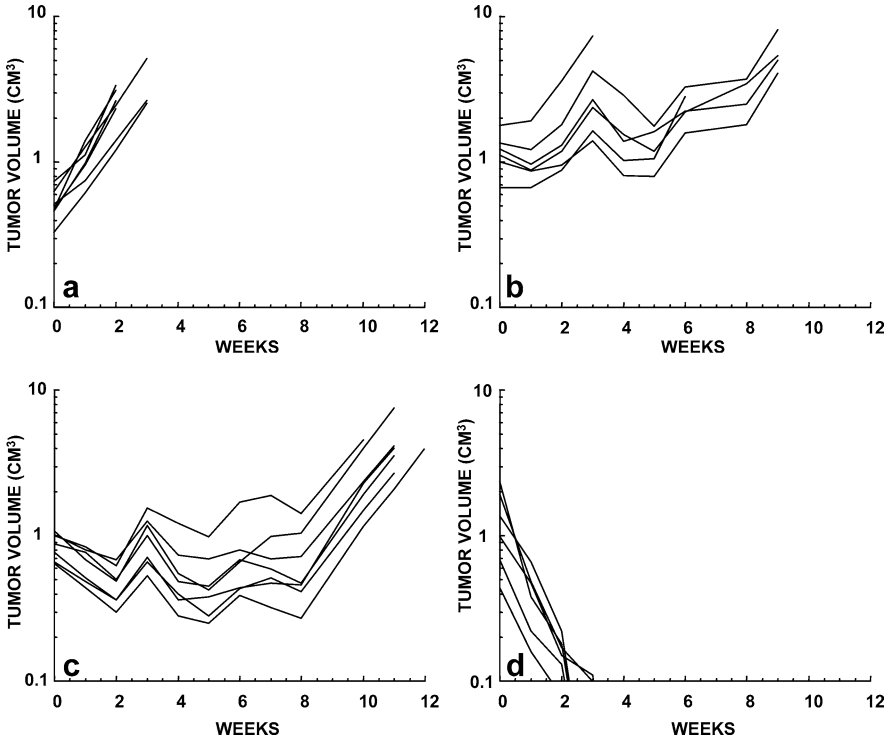

Fig. 2 Responses of SJ-BT27 PNET xenografts to single-agent irofulven, irinotecan or binary drug combinations. **a** Control; **b** irofulven 1.33 mg/kg; **c** irinotecan 0.4 mg/kg; **d** irofulven 1.33 mg/kg + irinotecan 0.4 mg/kg. Irofulven was administered intravenously [(d×5)1]3; irinotecan was administered intravenously [(d×5)2]3. Irofulven was administered during the first 5 days of irinotecan treatment in each cycle

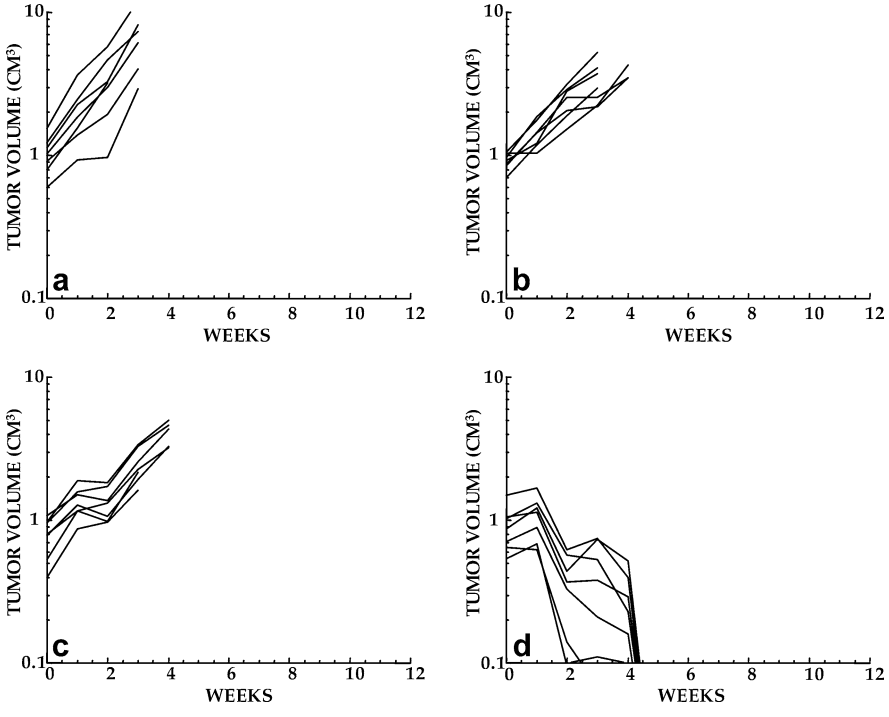

Fig. 3 Responses of NB-1771 neuroblastoma xenografts to single-agent irofulven, irinotecan or binary drug combinations. **a** Control; **b** irofulven 2.0 mg/kg; **c** irinotecan 0.4 mg/kg; **d** irofulven 2.0 mg/kg + irinotecan 0.4 mg/kg. Irofulven was administered intravenously [(d×5)1]3; irinotecan was administered intravenously [(d×5)2]3. Irofulven was administered during the first 5 days of irinotecan treatment in each cycle

CRs were maintained at 12 weeks for the 0.28 mg/kg and 0.4 mg/kg dose levels, respectively.

Discussion

Irofulven is a novel chemotherapeutic agent with activity against models of pediatric solid tumors; however, the plasma systemic exposure following administration as a single agent required to induce a significant rate of objective responses was in excess of that achievable in patients at tolerable doses [21]. Because of its unique spectrum of activity and the unlikely probability of producing cures as a single agent, there is value in developing irofulven in combination with other anticancer agents to improve treatment outcome. The purpose of this study was to determine the antitumor activity of irofulven administered in combination with

irinotecan in a series of tumor models that represent some of the most frequently occurring tumors in children. This combination is attractive because of relatively non-overlapping dose-limiting toxicities, myelosuppression for irofulven and diarrhea for irinotecan. In this study, the drugs were used individually, and in combination at suboptimal dose levels; doses were selected such that individual drugs would not produce CRs.

The combination treatment induced more CRs than did single agent irofulven or irinotecan therapy, and these responses were maintained for longer. However, after completing three cycles of treatment, tumor responses were only followed for an additional 4 weeks after the final dose of therapy; it is therefore unknown whether treatment was curative in these mice. Overall, the combination of irofulven and irinotecan generally showed improved activity over the individual agents in terms of time to tumor failure. Even in those tumor lines showing improvement with the combination therapy of only marginal statistical significance, striking biological responses were observed. Such examples included Rh18, DAOY, and SJ-BT29 xenografts, where responses were more durable in the combination therapy groups. The combination of irofulven and irinotecan had enhanced antitumor activity in seven of the eight human tumor xenografts.

The mechanism of cytotoxic action of irofulven is unknown, but the irofulven-induced DNA lesion(s) appear to inhibit transcription and DNA replication. Cells fully deficient in nucleotide excision repair, XPA, and specifically cells defective in transcription-coupled repair, CSA and CSB, were hypersensitive to irofulven, suggesting that the irofulven-induced DNA lesion stalls the replication fork in the transcribed strand, thus interfering with transcription. RAD18-deficient cells were also hypersensitive to irofulven, indicating that postreplication repair is inhibited and replication blockade cannot be overcome [13]. Although yet to be investigated, it is plausible that recombinational repair plays a role in cell survival from irofulven cytotoxicity, since RAD18-deficient cells show prolonged stalling of replication forks and increased frequency of recombination [22]. Agents that target topoisomerase I, such as irinotecan, can compromise recombinational processing of stalled replication forks. Thus irinotecan may sensitize cells to irofulven, and provide a basis for the interaction between irofulven and irinotecan.

When irofulven was administered as a single agent daily for 5 days with cycles repeated at 21-day intervals, a dose of 4.6 mg/kg was tolerated for up to three cycles. Using the same dosing schedule in the combination treatments, irofulven doses that ranged from 1.3 to 3.0 mg/kg were needed to yield significant objective response rates in the seven of the eight xenografts where the combination exhibited enhanced antitumor activity. This, however, corresponds with systemic exposures that range from fourfold to tenfold higher than that associated with the maximally tolerated dosage from the adult phase I clinical trial [3]. Nevertheless, it is possible that tumors exquisitely sensitive to irofulven (e.g., Rh30) may show objective responses at clinically tolerable systemic exposures. Having a better understanding of tumor biology may allow identification of patients who may tolerate and respond to irofulven therapy. For instance, irofulven has displayed preliminary clinical evidence of antitumor activity as monotherapy against platinum-resistant recurrent ovarian cancer [28]. Irinotecan also has shown some activity against ovarian cancer, and thus these patients may benefit from this combination. In addition, finding other chemotherapeutic agents to combine in a regimen with irofulven may allow the dose of irofulven to be even further decreased to a tolerable plasma exposure level.

In summary, our findings suggest a significant interaction between irofulven and irinotecan, such that suboptimal doses of each individual agent resulted in enhanced cytotoxicity. At this time, the mechanism of the combination activity remains unclear. The irofulven systemic exposure required to induce tumor responses in xenografts was greater than plasma systemic exposures reported at maximally tolerated irofulven dosages in patients. Because irofulven is active against aggressive tumors on which current chemotherapy has little impact, even tumors refractory to current therapies, pursuing alternative drug administration strategies and drug combinations is warranted.

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