Relation between Irofulven (MGI-114) Systemic Exposure and Tumor Response in Human Solid Tumor Xenografts

Markos Leggas, Clinton F. Stewart, Michael H. Woo, Maryam Fouladi, Pamela J. Cheshire, Jennifer K. Peterson, Henry S. Friedman, Catherine Billups, and Peter J. Houghton

INTRODUCTION

Irofulven (HMAF, MGI-114) is a semisynthetic anticancer agent derived from the sesquiterpene mushroom toxin illudin (1). This novel agent is less toxic than illudin S but is selectively cytotoxic toward malignant cells over normal cells (2, 3) with increased toxicity over other acylfulvene derivatives (3). Originally, drug development was targeted toward leukemias, because illudins were selectively cytotoxic to hematopoietic tumor cells over normal bone marrow progenitors (4). However, Irofulven has had great success in reducing or eliminating tumors in solid-tumor xenograft models (5–7) both in monotherapy and in combination with the topoisomerase I inhibitors irinotecan (8) and topotecan (4). In addition, Irofulven was very cytotoxic against adult and pediatric human tumors cultured in human tumor colony forming assays (9), including the refractory lung carcinoma line MV522 and a transfected multidrug-resistant positive MV522 line (9, 10). Illudins are in general quite cytotoxic to several lines exhibiting multidrug-resistant positive phenotypes (10–12). In vitro IC_{50} assays showed that effective illudin S concentrations ranged from the nanomolar to micromolar range, whereas the acylfulvene and HMAF derivatives were effective in the nanomolar to micromolar range (13–15).

In vitro studies showed that illudin S is rapidly uptaken into sensitive cells via a saturable energy-dependent mechanism, whereas nonsensitive cells showed a decreased illudin uptake, and no efflux of illudin or metabolites could be demonstrated (12). The mechanism responsible for the action of illudins and Irofulven is not well understood, but several pieces of evidence point toward apoptotic pathways after DNA damage and cell arrest in the G_{1}-S phase (14). Irofulven is a potent inhibitor of DNA synthesis and causes an S phase cell cycle arrest. Unlike other agents, it does not form DNA intrastrand cross-links or DNA-protein cross-links; however, brief exposure to Irofulven causes a limited number of DNA single-stranded breaks, with subsequent generation of small double-stranded fragments suggestive of apoptosis and subsequent cell death. This process is independent of de novo protein synthesis, as it can be abolished by folic acid.

ABSTRACT

Irofulven is a novel, small molecular weight semisynthetic compound, derived from a family of mushroom toxins known as illudins. This DNA alkylating agent has a chemical structure unlike any other chemotherapeutic agent in clinical use. The molecule is currently being studied in several Phase I, II, and III trials. The objectives of this study were to evaluate the antitumor activity of Irofulven in a panel of 20 pediatric solid tumor xenografts and to relate the Irofulven systemic exposure, defined as area under the concentration-time curve, to the antitumor dose associated with tumor regression in the tumor models. Irofulven was administered i.v. daily for 5 days with courses repeated every 21 days for a total of three cycles. The minimum effective dose of Irofulven causing objective regression (≥50% volume regression) of advanced tumors was determined for each of 19 of 20 independently derived tumor models (12 brain tumors, 4 neuroblastomas, and 4 rhabdomyosarcomas). At the maximum tolerated dose for three cycles of treatment (4.6 mg/kg/day) objective regressions were determined in 14 of 18 tumor lines (78%). However, the dose-response relationship was acute. At 2 mg/kg only 3 of 15 tumors tested demonstrated objective regressions, and in 3 additional tumors volume regressions were not achieved at a higher dose level (3 mg/kg), hence were not additionally tested. After administering the maximum tolerated dose (tolerated for one or two cycles of treatment) of Irofulven, 7 mg/kg, to mice bearing sensitive and resistant human tumors plasma concentration-time profiles were determined. Tumors were highly sensitive to Irofulven, but the systemic exposure required for a significant rate of objective response in this panel of tumors is in excess of that achievable in patients at tolerable doses, using this schedule of drug administration.

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in the presence of an endonuclease and apoptotic inhibitor such as zinc (14). Although the mechanism of Irofulven cytotoxicity remains undefined, it appears to be different from conventional chemotherapeutic agents. Irofulven inhibits growth of human cancer cells regardless of their p53 and p21 status (16), but repair of Irofulven-induced DNA damage may require the action of either ERCC2 or ERCC3 DNA helicase (11). Finally, HMAF and its cellular metabolites are likely to induce apoptosis by several different mechanisms including DNA damage, because they are highly bound and retained inside the cell for prolonged periods of time (15).

We have studied previously the efficacy of a number of drugs in the xenograft model and identified the minimum drug dose associated with antitumor effect (17–21). The relation between the minimum drug dose associated with antitumor effect and systemic exposure is then determined. The extent of systemic exposure associated with antitumor effects in the xenograft can then be compared with levels of systemic exposure reported at doses that are tolerated by patients. We have used these relationships to help prioritize new agents for clinical development and for the design of clinical protocols (22). At initiation of this study, the activity of Irofulven in pediatric solid tumors was unknown, as was the extent of plasma systemic exposure that would be necessary for an objective response in the xenograft model. The objectives of the present study were to evaluate the Irofulven antitumor activity in a panel of pediatric solid tumor xenografts and to relate the Irofulven systemic exposure, defined as the AUC, to the antitumor dose associated with tumor regression in the xenograft model. Moreover, the Irofulven systemic exposure related to tumor regression in the xenograft model was related to Irofulven systemic exposure (AUC) associated with tolerable doses in humans. These data may be valuable in understanding the utility of Irofulven in clinical trials of pediatric solid tumors.

MATERIALS AND METHODS

Immune Deprivation of Mice. Female CBA/CaJ mice (The Jackson Laboratory, Bar Harbor, ME) 4 weeks of age were immune-deprived by thymectomy, followed 3 weeks later by whole-body irradiation (1200 cGy) using a 137Cs source. Mice received $3 \times 10^6$ nucleated bone marrow cells within 6–8 h of irradiation. Tumor pieces of $\sim 3 \text{ mm}^3$ were implanted bilaterally in the space of the dorsal lateral flanks of the mice to initiate tumor growth. Tumor-bearing mice were randomized into groups of seven before therapy.

SCID Mice. CB17/Icr female SCID mice were implanted with a single tumor fragment, as described above. Tumor-bearing mice were randomized into groups of seven before therapy. All of the mice were maintained under barrier conditions. All of the experiments were conducted using protocols and conditions approved by the Institutional Animal Care and Use Committee.

Tumor Lines. Rhabdomyosarcoma lines (Rh12, Rh18, Rh28, and Rh30) have been described previously (22). All of the experiments were undertaken in tumors within 30 serial passages of being established in mice. Rh36 was established from a paratesticular embryonal rhabdomyosarcoma in a patient who had received dacarbazine, Adriamycin, and VAC (vincristine, actinomycin D, and cyclophosphamide) therapy. Drug evaluation was undertaken on passage 5. Neuroblastoma lines (NB-1771, NB-1382, NB-1643, and NB-1691) have been described previously (17). We also evaluated Irofulven against two medulloblastoma lines (DAOY and D283) and a glioblastoma line (SJ-GBM2), all of which have been described previously (23). In addition, nine newly established brain tumors were used in this evaluation (passages 2–13). The histological subtype for each is presented in Table 1. Full characterization of these xenografts will be presented elsewhere.

Growth Inhibition Studies. Mice bearing s.c. tumors each received the agent when tumors were approximately 0.20–1.0 cm in diameter. The procedures have been reported previously (23). Briefly, two perpendicular diameters were determined at 7-day intervals using digital Vernier calipers interfaced with a Macintosh computer. Tumor volumes were calculated assuming tumors to be spherical using the formula \( \left(\frac{\pi}{6}\right) \times d^3 \), where \( d \) is the mean diameter, and mice were followed for up to 12 weeks after starting treatment.

Tumor Response and Tumor Failure Time. For individual tumors PR was defined as a volume regression $>50\%$ but with measurable tumor $(\geq 0.10 \text{ cm}^3)$ at all times. CR was defined as a disappearance of measurable tumor mass $(<0.10 \text{ cm}^3)$ at some point within 12 weeks after initiation of therapy. Maintained complete response was CR without tumor regrowth within the 12-week study time frame. Where tumors were implanted in both dorsal flanks of each mouse in some of the xenograft studies, a mouse is said to achieve a CR only if tumors on both flanks have CRs, and a PR only if the tumor of at least one flank has a PR and the tumor response on the other flank is not worse than a PR. If an initial tumor volume was $<0.20 \text{ cm}^3$, data on that tumor was 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 before week 12 and before a tumor grew to four times its initial volume. In xenograft studies where tumors were implanted in both a large tumor, the tumor failure times from each mouse are clustered observations. Evidence of high correlation between failure times has been reported previously (23). Because the individual mouse is the unit of the experiment, the response of each mouse was taken to be the minimum of the two tumor failure times. This approach implicitly accounts for the clustering effect because of the mouse without explicitly specifying the correlation structure. For stud-
ies in which only one tumor was implanted in each mouse, response and tumor failure times were defined as above but no manipulation of the data was needed to find the minimum responses or times; individual data were used for those studies.

Statistical Methods. For comparisons of time to tumor failure for different treatment regimens, survival distributions of each treatment group were compared with the survival distribution of the control group using the exact log-rank test. Experiment-wise significance level was maintained at 0.05 by using the Bonferroni procedure to adjust for the multiplicity of tests of significance within each tumor line/study. SAS version 6.12 and StatXact-4 were used for statistical analysis.

Formulation and Administration. Irofulven as a dry powder was provided by MGI Pharma Inc. through the Cancer Drug Evaluation Program, National Cancer Institute or directly from MGI Pharma (Bloomington, MN). Irofulven (10 mg) was dissolved in 0.1 ml of absolute ethanol and after 5 min, 9.9 ml of 5% dextrose in water (5DW) were added. The drug was administered i.v. daily for 5 days. Cycles of treatment were repeated every 21 days for a total of three cycles of therapy.

Drug Administration and Sample Collection. The disposition of Irofulven was evaluated after a single administration of Irofulven at 3.0, 4.6, and 7 mg/kg by direct injection (duration of infusion <1 min) into a lateral tail vein. Pharmacokinetic studies were performed in nontumor-bearing CBA and SCID mice, and mice bearing SJBT29 (a sensitive ATRT) and SJBT33 (a resistant giant cell astrocytoma) tumors. Approximately 1 ml of blood was collected from three animals per time point before the dose and at 1, 5, 10, 15, 30, 60, 90, 120, and 150 min after i.v. administration of Irofulven. Blood samples were immediately centrifuged at 5500 × g for 2 min on a tabletop centrifuge. A portion of the separated plasma, 500 μl, was mixed into a 15-ml amber glass tube with 500 μl of 0.01 M sodium acetate buffer (pH 5.0). Samples were kept in dry ice for the duration of the experiment and were later stored at −80°C until the time of analysis.

Irofulven High-performance Liquid Chromatography Assay. Irofulven plasma concentrations were determined using the methods provided by MGI Pharma Inc. with minor modifications. Briefly, plasma was processed as described above, and 50 μl of internal standard solution (Illudin S; MGI Pharma; 2 mg/ml) were added to each sample followed by 0.7 g of sodium chloride, and 5 ml of pentane and dichloromethane (1:1). The samples were extracted for 20 min in a horizontal shaker and then centrifuged at 12,000 g for 30 min. The lower aqueous layer was then frozen by immersion into a dry ice-acetone bath, and the organic layer was decanted in a 7-ml amber vial and evaporated under a nitrogen stream at room temperature. The samples were reconstituted with 400 μl of mobile phase (15:25:60; methanol:acetonitrile:water) and transferred in 2-ml precolumned tubes for centrifugation (2 min at 12,000 × g). A 150 μl aliquot was injected using 100 μl injection loop onto an Altima C-18, 5 μm, 250 × 4.6 mm analytical column (Alltech Associates, Deerfield, IL) fitted with a Brownlee RP-18, 7 μm, 15 × 3.2 mm guard column (PerkinElmer, Norwalk, CT). The samples were eluted with the above mobile phase pumped at a flow rate of 1.0 ml/min with a Shimadzu LC-10AVP isocratic pump and were detected at 330 nm using a Shimadzu SPD-10AVP UV detector (Shimadzu Scientific Instruments, Columbia, MD). A nine-point calibration curve ranging from 5 to 20,000 ng/ml was used. All of the calibrators and controls for the xenograft studies were prepared in murine plasma (Hill Top Lab Animals Inc., Scottsdale, PA). Assay specificity was established using six different lots of blank murine plasma.

The intraday precision (coefficient of variation) was >95% for low (9 ng/ml) and high (4500 ng/ml) concentrations. The interday precision was >93%, and the accuracy was within 15%. The lower limit of quantitation was 5 ng/ml, and the linearity of the calibration curve as evaluated by the r² was >0.99.

Pharmacokinetic Analysis. Irofulven plasma concentration versus time data after i.v. administration was modeled using maximum likelihood estimation as implemented in ADAPT II version 4 (24). A two-compartment pharmacokinetic model was used, and model fits were evaluated by examination of residuals between predicted versus observed concentrations, correlation coefficients, and relative precision of parameter estimates. Parameters of the two-compartment model estimated included the volume of the central compartment (Vc), elimination rate constant (ke), and the intercompartment rate constants (kcp and kpc). Pharmacokinetic parameters that were calculated from these estimates included: systemic clearance (CL), volume of distribution at steady state (Vdss), and plasma AUC (25).

RESULTS

Efficacy Studies of Irofulven in Pediatric Tumor Xenografts. The studies presented were undertaken during a transition to SCID mice from immune-deprived CBA/CAj mice used previously in our studies. To compare antitumor responses in the two host systems, Irofulven was evaluated against Rh18, DA0Y, and SJ-BT37 tumors in both CBA and SCID mice. The antitumor activity of Irofulven was similar in both systems (data not presented), and CBA/CAj and SCID mice equally tolerated Irofulven. The maximum tolerated dose for a single 5-day course was ~7 mg/kg, whereas the maximum tolerated dose for up to three cycles of treatment (daily × 5 every 21 days) was ~4.6 mg/kg.

The antitumor activity of Irofulven is summarized in Table 2. Administered at 7 mg/kg daily for 5 days (three cycles of treatment over 7 weeks) Irofulven caused objective regressions in 14 (88%) of 16 tumor lines evaluated; however, in some experiments this dose was associated with a high lethality during and subsequent to the last cycle of treatment (~LD10). At 4.6 mg/kg, close to the LD10 dose level on this schedule, objective regressions were obtained in 14 of 18 (78%) tumor lines. However, antitumor activity diminished rapidly as the dose level was additionally reduced with objective regression in 8 of 19 (42%), 3 of 15 (14%), and 1 of 14 (7%) tumor lines at 3.0, 2.0, and 1.3 mg/kg, respectively (Table 2). Despite the low objective response rate at low dose levels, MGI-114 did cause significant retardation of growth in 4 tumor lines even at a dose of 1.3 mg/kg. Results for SCID-maintained Rh28, one of the most sensitive tumors, and SJ-BT40, one of the more resistant tumors, are presented in Figs. 1 and 2. The minimum dosage associated with objective regressions for each tumor model is summarized in Table 3.
Irofulven Pharmacokinetics after i.v. Administration. Depicted in Fig. 3 is the Irofulven plasma concentration-time profile after i.v. administration in mice bearing the resistant SJ-BT33 tumor line. The pharmacokinetic parameters for Irofulven in both nontumor and tumor-bearing mice are presented in Tables 4 and 5. The Irofulven systemic clearance and AUC0→∞ are similar for nontumor and tumor-bearing mice, suggesting that the disposition of the drug is unaffected by the presence of tumor. Irofulven plasma concentrations were greater than the limit of quantitation (5 ng/ml) for at least 2 h after drug administration in all of the experiments with tumor-bearing mice. In nontumor-bearing mice, Irofulven pharmacokinetics are linear (Cmax r² = 0.91; AUC r² = 0.83) over the dosage range examined. Because the presence of different tumors did not alter Irofulven disposition and the disposition was linear, systemic exposure (measured by AUC0→∞) associated with the lowest Irofulven dose achieving objective response for each xenograft line studied was extrapolated from the AUC measured after a dose of 7 mg/kg (data presented in Table 3).

DISCUSSION

Irofulven represents a new class of cytotoxic agent with significant antitumor activity against many preclinical tumor models. Presently we have evaluated this illudin S analogue against a spectrum of pediatric solid tumors maintained as xenografts in immune-deprived mice or SCID mice. Sensitivity of tumors was very similar in either mouse model, as was host toxicity. Irofulven was administered daily for 5 days with cycles repeated at 21-day intervals. A dosage of 7 mg/kg was tolerated for up to two cycles, whereas cumulative toxicity appeared to limit ability to administer a third cycle at this dosage level. Three cycles produced acceptable levels of toxicity at 4.6 mg/kg. Irofulven demonstrated broad-spectrum activity against neuroblastoma, rhabdomyosarcoma, and brain tumor xenografts. Complete or PRs were obtained in 14 of 16, 14 of 18, and 8 of 19 tumor lines at 7.0, 4.6, and 3.0 mg/kg, respectively. However, at lower dosage levels the response rate was far lower. Only 3 of 15 and 1 of 14 tumor models responded objectively (≥PR) at 2 mg/kg and 1.3 mg/kg.

Of interest was the observation that three of the six ATRT xenograft lines had a CR and the other three had a PR. These responses were observed at dosages between 3 and 7 mg/kg. ATRT is a distinct, highly aggressive clinicopathologic neoplasm, defined recently by histological, immunohistochemical, and cytogenetic criteria (26, 27). The lesion occurs primarily in children younger than 2 years (mean age at diagnosis, 17 months) and is often misdiagnosed as medulloblastoma. The outcome for patients with ATRT is uniformly dismal. Mean postoperative survival is only 11 months, with local recurrence and seeding of the cerebrospinal fluid pathways being common terminal events, despite aggressive chemotherapy. Since the definition of this entity, a significant percentage of infants diagnosed previously as medulloblastoma are being diagnosed with ATRT. Because Irofulven is remarkably active against mouse xenograft models bearing ATRT, it may be of significant potential benefit in the treatment of this highly aggressive tumor. However, accurate translation of such preclinical data requires the ability to achieve similar anticancer drug systemic exposure in patients consistent with that associated with that

Table 2 Antitumor activity of Irofulven against pediatric tumor xenografts

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Dose level (mg/kg)</th>
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<tbody>
<tr>
<td></td>
<td>7.0</td>
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<tr>
<td>DAOY</td>
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<tr>
<td>D283</td>
<td>++++++</td>
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<tr>
<td>SJ-BT12</td>
<td>ND</td>
</tr>
<tr>
<td>SJ-BT16</td>
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<td>ND</td>
</tr>
<tr>
<td>SJ-BT29</td>
<td>++++</td>
</tr>
<tr>
<td>SJ-BT33</td>
<td>-a</td>
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<tr>
<td>SJ-BT34</td>
<td>++++</td>
</tr>
<tr>
<td>SJ-BT36</td>
<td>++++</td>
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<tr>
<td>SJ-BT37</td>
<td>++++</td>
</tr>
<tr>
<td>SJ-BT40</td>
<td>+</td>
</tr>
<tr>
<td>SJ-GBM2</td>
<td>++++</td>
</tr>
<tr>
<td>NB-1771</td>
<td>ND</td>
</tr>
<tr>
<td>NB-1382</td>
<td>++++</td>
</tr>
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<td>NB-1643</td>
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<td>Rh28</td>
<td>++++</td>
</tr>
<tr>
<td>Rh30</td>
<td>ND</td>
</tr>
<tr>
<td>Rh36</td>
<td>++++</td>
</tr>
<tr>
<td>CR + PR/total</td>
<td>14/16</td>
</tr>
</tbody>
</table>

* Growth inhibition not significant (P > 0.05). All other results were significantly different from controls (P < 0.05).

* Response criteria: +++++++, CR maintained at week 12 (no regrowth by week 12); +++++, CR with regrowth by week 12; ++++, PR (≥50% regression); ++, growth stasis; +, growth inhibition equals 2 tumor-volume doubling times; +, growth inhibition equals 1 tumor-volume doubling time; −, no growth inhibition; and ND, not determined.
causing significant antitumor activity in the model system (28, 29).

Results of our pharmacokinetic studies in the xenograft model showed that Irofulven had a rapid distribution phase (5 min) and a longer elimination phase (approximately 50–100 min). The presence of tumor did not alter the Irofulven plasma clearance values (0.0166 versus 0.0195 or 0.0194 liter/hr/m²). Likewise, the Irofulven systemic exposure as measured by the AUC at a dose of 7 mg/kg was similar among the three groups and was 1152, 1011, and 1060 ng/ml/hr. We are not aware of published data regarding the disposition of Irofulven in mice, although it has been suggested that Irofulven has an extremely short elimination half-life in mice, rats, dogs, and monkeys from preclinical pharmacokinetic studies conducted by the manufacturer (30).

In a clinical trial Cox et al. (31) administered Irofulven to adults with advanced recurrent solid tumors by a 5-min infusion daily for 5 days every 28 days. In this Phase I trial, pharmacokinetic studies were performed in 46 patients studied with Irofulven dosages ranging from 1.0 to 17.69 mg/m². Pharmacokinetic studies were performed at each dosage level, and the parameters were similar between days 1 and 5, resulting in no accumulation of Irofulven during the dosing interval. This is not surprising because Irofulven had a rapid elimination half-life of 2–10 min. The mean (± SD) Irofulven Cmax values at the

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**Fig. 1** Responses of Rh28 rhabdomyosarcoma xenografts in SCID mice to Irofulven treatment. Irofulven was administered i.v. daily for 5 days. Courses of therapy were repeated every 21 days for a total of three cycles. A, control; B, 3.0 mg/kg; C, 2.0 mg/kg; and D, 1.32 mg/kg. Tumor volumes were determined every 7 days. Each curve represents growth of an individual tumor. E, relative tumor volumes for tumors in control (○), B, (●); C, (□); and D, (■). F, survival.

**Fig. 2** Responses of SJ-BT40 ATRT xenografts in SCID mice to Irofulven treatment. Irofulven was administered i.v. daily for 5 days. Courses of therapy were repeated every 21 days for a total of three cycles. A, control; B, 7.0 mg/kg; C, 4.6 mg/kg; D, 3.0 mg/kg; E, 2.0 mg/kg; and F, 1.32 mg/kg. Tumor volumes were determined every 7 days. Each curve represents growth of an individual tumor.

**Table 3** Minimum effective Irofulven dose associated with objective regressions of xenografts after i.v. administration

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Tumor lines</th>
<th>MGI AUC (ng/ml-h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3</td>
<td>Rh30</td>
<td>214</td>
</tr>
<tr>
<td>2.0</td>
<td>Rh28, DAOY</td>
<td>329</td>
</tr>
<tr>
<td>3.0</td>
<td>D283, SJ-BT27, SJ-BT34, SJ-BT36, NB1643</td>
<td>494</td>
</tr>
<tr>
<td>4.6</td>
<td>SJ-BT12, SJ-BT16, SJ-BT37, SJ-BT40, SJ-GBM2, NB-1382, Rh18</td>
<td>757</td>
</tr>
<tr>
<td>7.0</td>
<td>SJ-BT29, NB-1691, Rh36</td>
<td>1152</td>
</tr>
</tbody>
</table>

*a* Lowest dosage level evaluated.
recommended Phase II dosage of 10.64 mg/m² were 320 ± 219 ng/ml and 556 ± 226 ng/ml on days 1 and 5, respectively. The mean (± SD) Irofulven daily AUC values at this dosage were 33 ± 15 ng/ml·h and 50 ± 18 ng/ml·h·r, respectively. Moreover, they reported dose-proportional increases in \( C_{\text{max}} \) and AUC values among their patients. The dose-limiting toxicities were myelosuppression, primarily thrombocytopenia, and renal dysfunction, consistent with acute nonoliguric acute renal failure that promptly resolved. The authors reported one PR in a 60-year-old man with pancreatic cancer, and eight other patients maintained stable disease for three to five cycles of therapy. Other preliminary studies have reported occasional responses in pancreatic (32), prostate (33), and ovarian (34) carcinoma using different schedules of administration.

We administered Irofulven daily as a short infusion for 5 days as did Eckhardt et al. (30) in their Phase I clinical trial of Irofulven. Thirteen of our xenograft lines had CRs at Irofulven dosages of 1.3 (\( n = 1 \)), 2.0 (\( n = 2 \)), 3.0 (\( n = 3 \)), 4.6 (\( n = 4 \)), and 7.0 (\( n = 3 \)) mg/kg. Five xenograft lines had PRs at dosages of 4.6 (\( n = 4 \)) and 7.0 (\( n = 1 \)) mg/kg. At Irofulven dosages ranging from 1.3 to 7.0 mg/kg, the Irofulven daily systemic exposure ranged from 214 to 1152 ng/ml·h (see Table 3). This is compared with the Irofulven systemic exposure associated with the maximally tolerated Irofulven dosage from the adult Phase I clinical trial of Eckhardt et al. (30) 33 or 50 ng/ml·h·r (days 1 and 5). An important consideration when comparing Irofulven systemic exposures across species is the contribution of protein binding. The \textit{in vitro} serum protein binding shows that Irofulven is 80–82% bound in mice and 51–52% bound in man (over a concentration range from 500 to 1000 ng/ml). Although a slight difference in protein binding exists between species, it will not account for the >6-fold difference in plasma systemic exposure between that required for antitumor effect and that tolerated in the adult Phase I study.

At the lowest Irofulven dosage that was associated with an antitumor response in the xenograft model (\textit{i.e.}, 1.3 mg/kg), the Irofulven plasma systemic exposure was 6-fold greater than that tolerated in the adult Phase I study. Although in the adult Phase I study Irofulven has a rapid plasma half-life suggestive of metabolic conversion in blood or other tissues, no compelling data are available to show that a metabolite rather than the parent compound is responsible for the antitumor activity. Consequently, these results suggest that dose-limiting toxicities will limit the ability of patients to tolerate the extent of Irofulven systemic exposure that is associated with a high frequency of antitumor responses in the xenograft model. However, it is possible that infrequently tumors such as Rh30 may show objective responses at drug exposures achievable in patients. Understanding characteristics of such tumors may allow identification of patients who might more readily benefit from Irofulven therapy.

In summary, we have shown that Irofulven has broad and dramatic antitumor activity in a panel of pediatric solid tumors. Antitumor responses were noted in the xenograft model at Irofulven doses that were associated with acceptable toxicity in the xenograft model. However, Irofulven systemic exposure required to induce tumor responses in the xenograft are greater than plasma systemic exposures reported at maximally tolerated Irofulven dosage in patients. Because this compound is so highly active in the xenograft model against the highly aggressive ATRT tumor system we are pursuing alternative drug administration strategies. Moreover, we are also pursuing studies to confirm that the plasma Irofulven systemic exposure is a good surrogate for antitumor exposure by evaluating tumor extracellular fluid and tumor tissue Irofulven concentrations. By deriving preclinical data relating systemic exposure to antitumor activity in xenograft models, informed decisions might be made regarding the clinical development of new agents for the treatment of childhood and adult cancers.

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4. S. Waters, personal communication.
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REFERENCES


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