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ORIGINAL ARTICLE

Toxicology and pharmacokinetic study of orally administered 5-iodo-2-pyrimidinone-2′deoxyribose (IPdR) × 28 days in Fischer-344 rats: impact on the initial clinical phase I trial design of IPdR-mediated radiosensitization

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Abstract
Purpose A toxicology and pharmacokinetic study of orally administered (po) IPdR (5-3iodo-2-pyrimidinone-2′deoxyribose, NSC-726188) was performed in Fischer-344 rats using a once daily (qd) × 28 days dosing schedule as proposed for an initial phase I clinical trial of IPdR as a radiosensitizer.
Methods For the toxicology assessment, 80 male and female rats (10/sex/dosage group) were randomly assigned to groups receiving either 0, 0.2, 1.0 or 2.0 g $kg^{-1}day^{-1}$ of po IPdR × 28 days and one-half were observed to day 57 (recovery group). Animals were monitored for clinical signs during and following treatment with full necropsy of one-half of each dosage group at day 29 and 57. For the plasma pharmacokinetic assessment, 40 rats (10/sex/dosage group) were randomly assigned to groups receiving either 0.2 or 1.0 g $kg^{-1}day^{-1}$ of po IPdR × 28 days with multiple blood samplings on days 1 and 28 and single blood sampling on days 8 and 15.
Results No drug-related deaths occurred. Higher IPdR doses resulted in transient weight loss and transient decreased hemoglobins but had no effect on white cells or platelets. Complete serum chemistry evaluation showed transient mild decreases in total protein, alkaline phosphatase, and serum globulin. Necropsy evaluation at day 29 showed minimal to mild histopathologic changes in bone marrow, lymph nodes and liver; all reversed by day 59. There were no sex-dependent differences in plasma pharmacokinetics of IPdR noted and the absorption and elimination kinetics of IPdR were found to be linear over the dose range studied.
Conclusions A once-daily dosing schedule of po IPdR for 28 days with doses up to 2.0 g $kg^{-1}day^{-1}$ appeared to be well tolerated in Fischer-344 rats. Drug-related weight loss and microscopic changes in bone marrow, lymph nodes and liver were observed. These changes were all reversed by day 57. IPdR disposition was linear over the dose range used. However, based on day 28 kinetics it appears that IPdR elimination is enhanced following repeated administration. These toxicology and pharmacokinetic data were used when considering the design of our initial phase I trial of po IPdR as a clinical radiosensitizer.

Keywords IPdR · Radiosensitization · PK · Toxicology

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Introduction

5-iodo-2-pyrimidinone-2′-deoxyribose (IPdR) is a pyrimidinone nucleoside which was originally synthesized as an antiviral agent based on the hypothesis that nucleosides without an amino- or keto- group at position 4 of the pyrimidine ring would be used as a substrate of viral thymidine kinase (TK) but not mammalian TK [5, 12]. However, in early in vivo experimental studies by these same investigators, it was found that IPdR was efficiently

converted to 5-iodo-2′-deoxyuridine (IUdR) by an aldehyde oxidase primarily localized to the liver in rodents [4]. The spectrum of pro-drug metabolism by hepatic aldehyde oxidase was recently reviewed [14].

IUdR is a halogenated thymidine analog which has been recognized as an in vitro/in vivo and potential clinical radiosensitizer for nearly 50 years (reviewed in 5). IUdR is converted intracellularly to IdUMP by TK and sequentially phosphorylated to IdUTP, where it competes for DNA incorporation with dTTP. The biochemical mechanism of radiosensitization is related to the enhanced generation of highly reactive free radicals by ionizing radiation (IR) from IUdR-DNA incorporation resulting in enhanced IR-induced DNA double strand breaks (DSBs) while also altering IR-DNA damage repair [6]. We and others have demonstrated in experimental in vitro and in vivo human tumor models that the %IUdR-DNA incorporation correlates directly with the extent of radiosensitization [6]. We have also demonstrated that both DNA mismatch repair (MMR) and base excision repair (BER) can repair (remove) IUdR-DNA mismatches (particularly G:IU), suggesting that MMR- and/or BER-deficient tumors might be selectively targeted for IUdR-mediated radiosensitization [1, 2, 21, 22, 25]. However, a major drawback of IUdR as a clinical radiosensitizer is that it requires prolonged exposures (typically by continuous intravenous infusions), which results in enhanced IUdR-DNA incorporation in rapidly proliferating normal tissues with myelosuppression (principally neutropenia and less often thrombocytopenia) and acute gastrointestinal toxicities (principally diarrhea and liver function test abnormalities) as observed in phase I and II clinical trials of infusional IUdR [3, 7, 15, 18, 20, 23, 24]. For this reason, primary or metastatic tumors of the brain and high-grade soft tissue/bone sarcomas are often selected as clinical targets for IUdR-mediated radiosensitization as these tumor types are typically surrounded by non-proliferating normal tissues. While the results of these clinical phase I and II trials suggest some effect on IUdR-mediated radiosensitization, systemic acute toxicities to bone marrow and intestine still limited the duration and total dose of continuous infusions of IUdR administered for 7–14 days [3, 7, 18, 20, 23, 24]. Our recent phase I trial using a 28 day continuous intravenous infusion of IUdR immediately before and during irradiation demonstrated increased survival in patients with anaplastic astrocytomas confirming two prior IUdR trials of shorter IUdR infusion schedules [20, 24], but the IUdR dose escalation was again limited by systemic bone marrow and gastrointestinal toxicities [15].

In the development of a radiosensitizer such as IUdR, one basic premise is that IUdR should be administered prior to beginning radiation therapy and then continued during treatment as tumor cell repopulation has been demonstrated to occur within a few weeks of initiating radiation therapy [6]. Thus, a prolonged exposure is theoretically necessary to enhance IUdR-mediated radiosensitization. Over the last decade, our laboratory group has been involved in the pre-clinical development of po IPdR as a prodrug for IUdR-mediated radiosensitization in human tumors. In a series of in vivo experimental studies over the last 12 years [8–11, 16, 17], we have determined the pharmacokinetics (PK) of po IPdR and its active metabolite IUdR in rodents and monkeys [8–10] and demonstrated an improved therapeutic gain for IUdR-mediated radiosensitization for po IPdR compared to continuous infusion IUdR (7–14 day exposures), using subcutaneous human colon cancer and human glioblastoma xenografts in athymic mice [8, 9, 11, 16, 17]. Additionally, we reported an efficient in vitro conversion of IPdR to IUdR using cytosolic extracts from normal human liver specimens, suggesting that normal human liver has significant IPdR-aldehyde oxidase activity, which we found to be markedly lower (>1 log) in cytosolic extracts from normal human small intestines [9]. Importantly, in our experimental in vivo studies in athymic mice with human tumor xenografts, we also found much lower levels of IUdR-DNA incorporation in normal bone marrow and normal intestine using po IPdR compared to continuous infusion IUdR [8–11, 17].

More recently, we have worked with the National Cancer Institute (NCI) through a RAID grant (#197) to complete the pre-clinical development of po IPdR as a radiosensitizer in order to obtain an investigator-initiated IND from the Food and Drug Administration (FDA) to begin clinical testing of po IPdR. As our initial phase I clinical trial design plans to use a once daily (qd) × 28 day dosing schedule of IPdR to compare results to our recently completed phase I trial of continuous intravenous infusions of IUdR × 28 days [15], the FDA requested this full toxicology and pharmacokinetic study. The details of this study design for both toxicology and pharmacokinetic endpoints were collaboratively reviewed by NCI and FDA officials as part of the RAID grant.

Materials and methods

Drugs and chemicals

IPdR (NSC-726188) was synthesized at Ash-Stevens Pharmaceuticals (Detroit, MI) under the RAID grant and provided to the Battelle Memorial Institute (Columbus, OH) for this toxicology and pharmacokinetic study (Lot: PKS-10G-125). The vehicle was 0.5% carboxymethyl cellulose in milli-Q deionized water (Sigma-Aldrich, St Louis, MO). The dose volume for daily gavage for control

and treatment groups in both the toxicology and pharmacokinetic arms of this study was 10 ml kg^{-1}.

Animals and study groups

Nine to 10 week-old male and female Fischer 344 rats (Taconic, Germantown, NY) were used for this study. Body weights ranged from 184.8 to 268.8 g for male rats and from 121.5 to 153.5 g for female rats on day 1 of the study. For the toxicology arm of the study, a total of 80 male and female rats were randomly assigned to either a vehicle control (0 g $kg^{-1}day^{-1}$ IPdR) group or to one of three IPdR dose groups (0.2, 1.0 or 2.0 g $kg^{-1}day^{-1}$) with an equal number of males and females in each of the four groups. These rats were dosed by oral gavage for 28 consecutive days and one-half of each dose group were necropsied at day 29 and the other half on day 57 (recovery group). For the pharmacokinetic arm of the study, a total of 40 rats (10/sex/group) were randomly assigned to receive either 0.2 or 1.0 g $kg^{-1}day^{-1}$ of IPdR po for 28 days. These animals were gavaged dosed with IPdR and two rats/sex/time point had blood samples collected on study days 1, 8, 15 and 28 at the specified times for IPdR and IUdR plasma analyses as described below.

For both arms of the study, rats were individually housed in polycarbonate cages and provided a certified rodent diet (Harlan 2018C) and water ad libitum. Rodent housing and feeding guidelines conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the US Department of Agriculture through the Animal Welfare Act (Public Law 99–198).

Toxicology study

Animals were observed at least once daily and weighed every 3–4 days during the IPdR dosing period and then weekly to day 57. Blood samples were collected from rats on the toxicology arm of the study on days 0, 8, 15, 29, 43 and 57. A CBC including differential and a comprehensive serum chemistry panel (BUN, creatinine, glucose, electrolytes and liver function tests) were measured at each time point. For the toxicology arm, five male and five female rats from each group underwent full necropsy on either day 29 or day 57 of the study. Body weights and clinical pathology samples were taken on the day of necropsy. Both gross and microscopic evaluations of all organs/tissues were performed on each study rat at the day 29 or day 57 necropsy by a certified vetinary pathologist. The gross tissues were examined, sampled and then fixed in 10% neutral buffered formalin. Hematoxylin and eosin stained sections of all organs/tissues were examined microscopically and compared to the gross pathology records. All pathologic findings were categorized either as drug-related or nondrug-related. Each pathologic finding was coded by the most specific topographic and morphologic diagnoses, severity, and distribution using the Pathology Terminology Guidelines of the Toxicology Data Management System (TDMS) for the National Toxicology Program (July 1992). A four grade scoring system of microscopic findings described as minimal, mild, moderate or severe was used.

Pharmacokinetic study

Blood samples for the pharmacokinetic arm of the study were collected on days 1 and 28 at 15, 30, 60 and 90 min and 2, 4, 6, 8, 12 and 24 h after IPdR dosing. Approximately 1–1.5 ml of blood was collected from the retro-orbital plexus using capillary tubes into heparinized containers according to the above time schedule. Each rat was bled twice, once at two different time points on both days 1 and 28. The 24 h sample on day 29 was a terminal sample. In addition, rats were bled prior to IPdR dosing on days 8 and 15 to determine whether steady-state plasma concentrations were achieved. Blood was separated into plasma using centrifugation and the plasma was stored at –20°C until analysis for IPdR and IUdR. A sensitive liquid chromatography with tandem mass spectrometry detection (LC–MS/MS) method was established to measure IPdR and IUdR plasma levels over a concentration range of 0.05–2.0 μg^{-1} mL (i.e., 0.148–5.92 μM for IPdR and 0.141–5.65 μM for IUdR).

Plasma pharmacokinetic analyses of IPdR and IUdR

The IPdR and IUdR plasma concentration time profiles were evaluated using compartmental analysis modules and non-compartmental analysis (NCA) modules, respectively, in WinNonlin® (version 4.0.1, Pharsight Corporation Mountain View, CA). Individual concentrations were used in the compartmental analysis of IPdR data and the time points used to construct the plasma concentration data sets were actual time points. Alternatively, the mean value of two concentrations and the target time points were used in the NCA for IUdR data sets.

For the IPdR compartmental analysis, a one-compartment model with no lag phase and first order absorption and elimination was used as follows:

Using the equation:

$$C(T) = \frac{DFk_{01}}{V(k_{01} - k_{10})}(\exp(-k_{10}T) - \exp(-k_{01}T))$$

where

$C(T)$ = concentration at time (t)
D = dose
F = oral bioavailability
V = volume of distribution
k_{01} = absorption rate constant
k_{10} = elimination rate constant

The area under the curve (AUC) values for IPdR were calculated to infinity from the model prediction.

When evaluating the IUdR pharmacokinetic profiles using NCA, the values used to define the terminal linear phase were automatically identified by the WinNonlin® algorithm, which uses a linear trapezoidal method with linear interpolation for AUC calculation. These points were used to estimate the first-order rate constant associated with the terminal linear phase, Lambda Z (λZ), by performing a regression of the natural logarithm of the concentrations on sampling time, for those times in the above specified time range. Lambda Z is equal to the absolute value of the estimated slope.

Results

Toxicology study

Drug-related clinical signs of hunched posture, rough coat or thin appearance were recorded for several animals dosed at 0.2 g $kg^{-1}day^{-1}$ and higher with the number of affected animals being IPdR dose-dependent including 18 of 20 rats (10 male/8 female) in the 2.0 g $kg^{-1}day^{-1}$ group with at least one clinical sign. However, all male and female rats in the 0.2 and 1.0 g $kg^{-1}day^{-1}$ groups and females in the 2.0 g $kg^{-1}day^{-1}$ group gained weight throughout drug treatment at a rate comparable to the vehicle control animals. Males receiving 2.0 g $kg^{-1}day^{-1}$ exhibited mean body weight decreases of 8–11% beginning on day 5 of drug treatment; this was reversed (±5%) by day 36 of study. One female rat receiving IPdR at 1.0 g $kg^{-1}day^{-1}$ died unexpectedly on study day 23 from acute pulmonary hemorrhage consistent with gavage-induced trauma. No drug-related deaths occurred.

Analyses of the clinical pathology parameters showed a decrease in mean values of red cell counts (RBC), hemoglobin (Hgb) and hematocrit (Hct) in both males and females in the 1.0 and 2.0 g $kg^{-1}day^{-1}$ dose groups, most marked at day 15 compared to either day 8 or day 29 values. An increased percent reticulocyte count and greater mean corpuscular volume (MCV) were noted in the two higher IPdR dose groups at days 15 and 29. No other differences in hematologic parameters between the vehicle control and IPdR drug-treated groups were found, supporting our prior observations of low levels of %IUdR-DNA incorporation in circulating granulocytes in mice with daily IPdR dosing for 7 or 14 consecutive days compared to the %IUdR-DNA incorporation in mouse circulating granulocytes using maximum tolerated doses (MTD) of continuous infusion IUdR [8, 9, 11]. Additionally, rats receiving 1.0 and 2.0 g $kg^{-1}day^{-1}$ dosings showed mild decreases in mean serum globulin, alkaline phosphatase and total protein with mild increases in serum albumin and alanine aminotransferase on days 8, 15, and 29 with full recovery by day 42. No other effects on serum chemistries were noted including normal values for hepatic transaminases, BUN, creatinine and electrolytes throughout the entire study period.

By study design, one-half of the rats from each dosing group on the toxicology arm underwent complete necropsy at day 29 (immediately following the 28-day drug schedule), while the other half of rats underwent complete necropsy after a 28-day observation period following drug treatment (day 57). At necropsy on day 29, only one rat (male receiving 2.0 g $kg^{-1}day^{-1}$) had a drug-related gross pathologic finding, a small thymus gland, which correlated microscopically with thymic lymphoid depletion (cortex thinning) in this rat. This pathology was found microscopically in several other rats in the highest IPdR dose group. Other drug-related microscopic findings in day 29 necropsied rats were noted in bone marrow (marrow erythrocyte hyperplasia), some lymphoid tissues (such as lymphoid depletion in the thymus as well as in some mandibular and mesenteric lymph nodes), liver (hepatocyte cytoplasmic vacuolization) and salivary glands (atrophy of serous and mucinous glands in the mandibular gland). These drug-related microscopic findings in rats sacrificed on day 29 were typically graded as minimal to mild with an occasional moderate grade. No severe grades were recorded. All drug-related microscopic findings appeared to recover within 4 weeks of post-drug observation based on the analysis of rats necropsied on day 57, except for some residual salivary gland atrophy, which was mostly graded as minimal. Non-drug related microscopic changes were typical of incidental developmental or inflammatory changes that are commonly seen in laboratory rats of this age and strain. As mentioned previously, one non-drug related death occurred from gavage-induced trauma resulting in acute pulmonary hemorrhage.

Day 1 IPdR pharmacokinetic analysis

The plasma concentration-time curves for IPdR using the day 1 observed values and fitted curves are shown in Fig. 1 for male and female rats at the two IPdR doses used, 1,200 mg m^{-2} day (0.2 g $kg^{-1}day^{-1}$) (plate A) and 6,000 mg m^{-1} day (1.0 g $kg^{-1}day^{-1}$) (plate B), respectively. The IPdR pharmacokinetic parameters for both day 1 and day 28 are presented in Table 1 (male rat dosage groups) and II (female rat dosage groups). These IPdR plasma concentration-time curves in Fig. 1 are best described by a one-compartment model with first order absorption and elimination. IPdR was absorbed slowly following gastric gavage of a fixed volume (10 $ml^{-1}kg$) into the systemic circulation with absorption half-life (k01-HL) values of 60 and 44 min for male and female 1,200 mg m^{-2} dosage groups and 27 and 30 min for male and female 6,000 mg kg dosage groups, respectively (Tables 1 and 2). However, if the standard errors of the estimates are taken into consideration, there were no apparent sex- or dose-dependent differences in absorption half-life. The observed C_{max} values were 26.6 and 32.3 μM for male and female 1,200 mg m^{-2} groups and 110 and 113 μM for the male and female 6,000 mg m^{-2} groups, respectively. These observed C_{max} values were larger than the predicted C_{max} values but both parameters suggested a less than dose-proportional increase in C_{max}.

The observed T_{max} values were 120 min for all groups except for the female 1,200 mg m^{-2} group, which was 90 min (Tables 1 and 2). The predicted T_{max} values were similar for all groups and ranged from 103 to 115 min, suggesting no IPdR dose- or sex-dependent differences in T_{max}. The apparent volume of distribution (*V*/*F*) was 68 and 114 L m^{-2} for the male and female 1,200 mg m^{-2} groups, and 278 and 247 L m^{-2} for the male and female 6,000 mg m^{-2} groups. There was no sex-dependent difference in *V*/*F* compared to the same dosage groups but lack of information on bioavailability (*F*) prevented appropriate comparison of *V*/*F* across the two dosage groups.

The elimination half-life (k_{10}-HL) increased with dose but was not sex-dependent (Tables 1 and 2). The male and female half-life values were 109 and 127 min in the male and female 1,200 mg m^{-2} dosage groups and 421 and 323 min for the male and female 6,000 mg m^{-2} dosage groups, respectively; representing a threefold to fourfold increase in the elimination half-life in these low-to-mid IPdR dosage groups. Although the apparent clearance (CL_F) values were similar for male and female rats in both IPdR dose groups when the variability of the parameter values was taken into consideration (i.e., values ranged from 0.433 to 0.624 L $min^{-1}m^{-2}$), it was not possible to determine whether clearance was dose-dependent for the same reason as cited above for *V*/*F*.

The AUC values were similar between sexes considering the variability associated with the parameter. For the 1,200 mg m^{-2} group, the male and female AUC values were 8,207 and 5,690 μM min, whereas for the 6,000 mg m^{-2} group, the male and female AUC values were 38,800 and 33,400 μM min^{-1}, respectively. The increases in AUC values in both male and female rats approximated (within 5–15%) a dose-proportional increase.

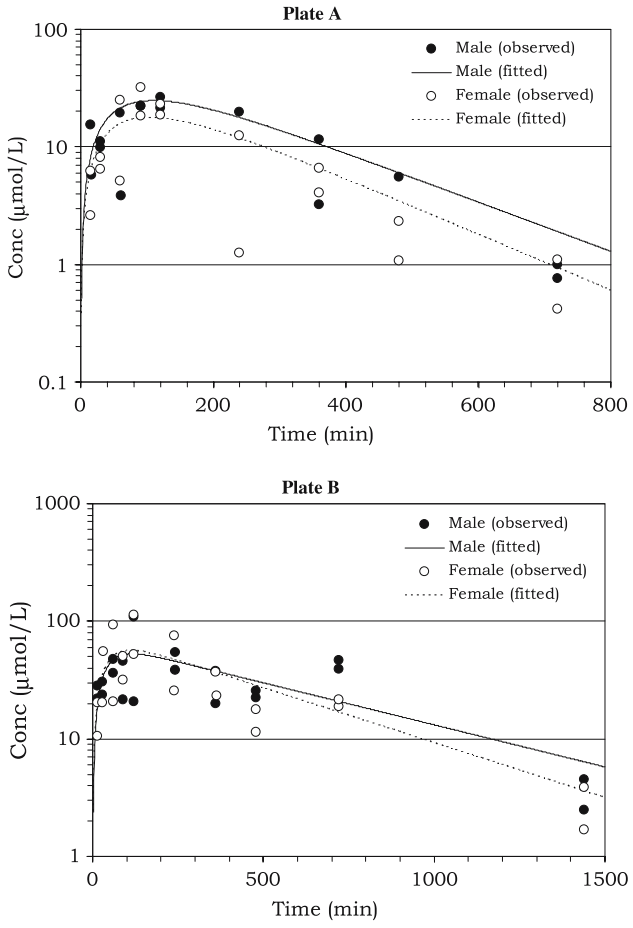

Fig. 1 IPdR plasma concentration time curves for male and female Fischer-344 rats following a single gavage administration (day 1) of IPdR at dosages of 1,200 mg m^{-2} (Plate A) or 6,000 mg m^{-2} (Plate B)

Day 28 IPdR pharmacokinetic analysis

The plasma concentration-time curves for the day 28 IPdR pharmacokinetic analysis are shown in Fig. 2 for male rats and female rats for the 1,200 mg m^{-2} day^{-1} (0.2 g $kg^{-1}day^{-1}$) (plate A) and 6,000 mg m^{-2} day^{-1} (1.0 g $kg^{-1}day^{-1}$) (plate B) dose groups, respectively. The day 28 pharmacokinetic (PK) parameters are also listed in

Table 1 Pharmacokinetic (PK) parameters for IPdR based on one-compartment model analyses of concentration time curves for male Fischer-344 rats after a single (day 1) and multiple daily (day 28) Gavage administration(s) of IPdR

PK parameter	Unit	Male rat (1,200 mg m^{-2}, day 1)			Male rat (6,000 mg m^{-2}, day 1)			Male rat (1,200 mg m^{-2}, day 28)			Male rat (6,000 mg m^{-2}, day 28)		
		Estimate	SE	%CV	Estimate	SE	%CV	Estimate	SE	%CV	Estimate	SE	%CV
V/F	L m^{-2}	68.02	27.74	41	277.8	64.0	23	97.73	16.78	17	347.6	50.2	14
k_{01}	L min^{-1}	0.01150	0.00612	53	0.02550	0.01254	49	0.03219	0.01170	36	0.06887	0.03728	54
k_{10}	L min^{-1}	0.006359	0.002046	32	0.001647	0.000375	23	0.005850	0.000587	10	0.002066	0.000289	14
AUC	μM min^{-1}	8,207	1,087	13	38,780	6,030	16	6,208	642	10	24,710	2,690	11
k_{01}_HL	min	60.26	32.01	53	27.18	13.35	49	21.53	7.82	36	10.06	5.44	54
k_{10}_HL	min	109.0	35.0	32	420.8	95.8	23	118.5	11.9	10	335.4	46.8	14
CL/F	L min^{-1} m^{-2}	0.4325	0.0573	13	0.4576	0.0712	16	0.5718	0.0592	10	0.7183	0.0783	11
T_{max}	min	115.2	18.4	16	114.9	35.7	31	64.74	13.38	21	52.49	20.56	39
C_{max}	μM	25.08	3.23	13	52.86	8.85	17	24.87	2.86	12	45.81	5.68	12

Abbreviations used, *V/F* apparent volume of distribution; k_{01} absorption rate constant, k_{10}, elimination rate constant; *AUC* area under the curve, k_{01}_HL, absorption half-life; k_{10}_HL, elimination half-life; CL/F, apparent clearance; T_{max} maximum time; C_{max} maximum concentration

Table 2 Pharmacokinetic (PK) parameters for IPdR based on one-compartment model analyses of concentration time curves for female Fischer-344 rats after a single (day 1) and multiple daily (day 28) Gavage administration(s) of IPdR

PK parameter	Unit	Female rat (1,200 mg m^{-2}, day 1)			Female rat (6,000 mg m^{-2}, day 1)			Female rat (1,200 mg m^{-2}, day 28)			Female rat (6,000 mg m^{-2}, day 28)		
		Estimate	SE	%CV	Estimate	SE	%CV	Estimate	SE	%CV	Estimate	SE	%CV
V/F	L m^{-2}	113.8	35.6	31	247.3	52.4	21	133.1	22.8	17	233.6	99.6	43
k_{01}	L min^{-1}	0.01561	0.00742	48	0.02312	0.01005	43	0.04139	0.01613	39	0.02667	0.01572	59
k_{10}	L min^{-1}	0.005481	0.001254	23	0.002149	0.000355	17	0.004879	0.000606	12	0.007073	0.004175	59
AUC	μM min^{-1}	5,690	790	14	33,380	4,450	13	5,464	534	10	10,740	2,400	22
k_{01}_HL	min	44.42	21.10	48	29.98	13.02	43	16.74	6.52	39	25.99	15.30	59
K_{10}_HL	min	126.5	28.9	23	322.5	53.3	17	142.1	17.6	12	98.00	57.79	59
CL/F	L min^{-1} m^{-2}	0.6237	0.0866	14	0.5316	0.0709	13	0.6496	0.0636	10	1.653	0.370	22
T_{max}	min	103.4	20.8	20	113.3	30.3	27	58.55	13.74	23	67.73	12.19	18
C_{max}	μM	17.70	2.63	15	56.25	8.38	15	20.03	2.38	12	47.04	6.03	13

Abbreviations used: see Table 1

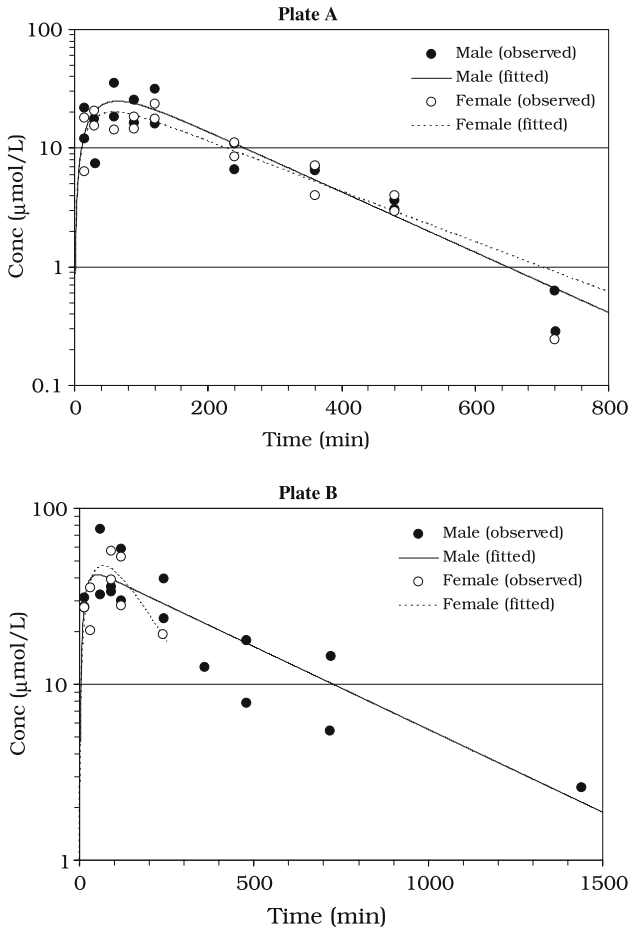

Fig. 2 IPdR plasma concentration time curves for male and female Fischer-344 rats following multiple daily gavage administrations (day 28) of IPdR at dosages of 1,200 mg m^{-2} (Plate A) or 6,000 mg m^{-2} (Plate B)

Table 1 (male rats) and II (female rats). IPdR absorption remained slow with the absorption half-life (k_{01}_HL) values of 22 and 17 min for male and female 1,200 mg m^{-2} day^{-1} dose groups, and 10 and 26 min^{-1} for male and female 6,000 mg m^{-2} day^{-1} dose groups. No dose- or sex-dependent differences in absorption half-life values were apparent. While the day 28 values are lower than day 1 values, the variability in the parameter estimates suggested no difference in absorption half-life values following single (day 1) and multiple IPdR administrations (day 28). The observed C_{max} values for the male and female 1,200 mg m^{-2} day^{-1} groups were 35.3 and 23.7 μM, and for the male and female 6,000 mg m^{-2} day^{-1} groups were 76.6 and 57.3 μM, suggesting a less than dose proportional increase in C_{max}, which was a consistent outcome using the predicted C_{max} values. The observed T_{max} values ranged from 60 to 120 min for all groups and the predicted T_{max} values were similar for all groups, ranging from 52 to 68 min. Overall, there were no sex-dependent differences in the C_{max} and T_{max} parameters.

The apparent volume of distribution (V/F) was 97.7 and 133 L m^{-2} day^{-1} for the male and female 1,200 mg m^{-2} day^{-1} groups, and 348 and 234 L m^{-2} day^{-1} for the male and female 6,000 mg m^{-2} day^{-1} groups, respectively. No sex-dependent differences were found on day 28 V/F values and no significant differences in V/F were found comparing day 1 and day 28 groups. The elimination half-life (k_{10}-HL) values increased approximately 2.8-fold (119 to 335 min) in males with an increased IPdR dosage, but no valid comparison was possible in female rats as the late (360 and 720 min) time points in the 6,000 mg m^{-2} day^{-1} group were not reliable. Although apparent clearance (CL/F) values were similar in both low and mid-dose male rat groups, no dose-dependent comparison could be adequately made.

The day 28 IPdR AUC values were considered similar between sexes. For the 1,200 mg m^{-2} day^{-1} dose groups, the male and female AUC values were 6,210 and 5,460 μM min^{-1}; whereas for the 6,000 mg m^{-2} group the male and female AUC values were 24,700 and 10,700 μM min^{-1}, respectively. A less than dose proportional increase in AUC values was evident in males and females, although the uncertainty in the terminal linear phase for female 6,000 mg m^{-2} day^{-1} rats affected the validity, similar to the elimination half-life comparison cited above.

Days 8 and 15 IPdR pharmacokinetic analysis

The pre-dose plasma IPdR concentrations (C_{min}) on both days 8 and 15 were below the limit of quantitation for the 1,200 mg m^{-2} day^{-1} dose groups but measurable in the 6,000 mg m^{-2} day^{-1} rats. The mean (± SD) plasma (C_{min}) concentrations in the 6,000 mg m^{-2} day^{-1} dose group on day 8 were 2.84 ± 1.15 μM for males and 2.00 ± 1.42 μM for females compared to day 15 values of 1.15 ± 1.08 μM for males and 0.843 ± 0.938 μM for females.

Day 1 IUdR pharmacokinetic analysis

The plasma concentration-time curves for IUdR are illustrated in Fig. 3 and the IUdR pharmacokinetic parameters, based on non-compartmental analysis (NCA), are summarized in Table 3 (males) andTable 4 (females). There were no apparent IPdR dose- or sex-dependent differences in the observed C_{max} and T_{max} values. A less than dose proportional increase in observed C_{max} was found with the C_{max} values being 2.69 and 2.85 μM for male and female 1,200 mg m^{-2} groups compared to 2.65 and 4.30 μM for the male and female 6,000 mg m^{-2} groups, respectively, based on the highest average concentration for the group. The observed T_{max} values were 15 min for all groups, except for the 6,000 mg m^{-2} males. A comparison of the day

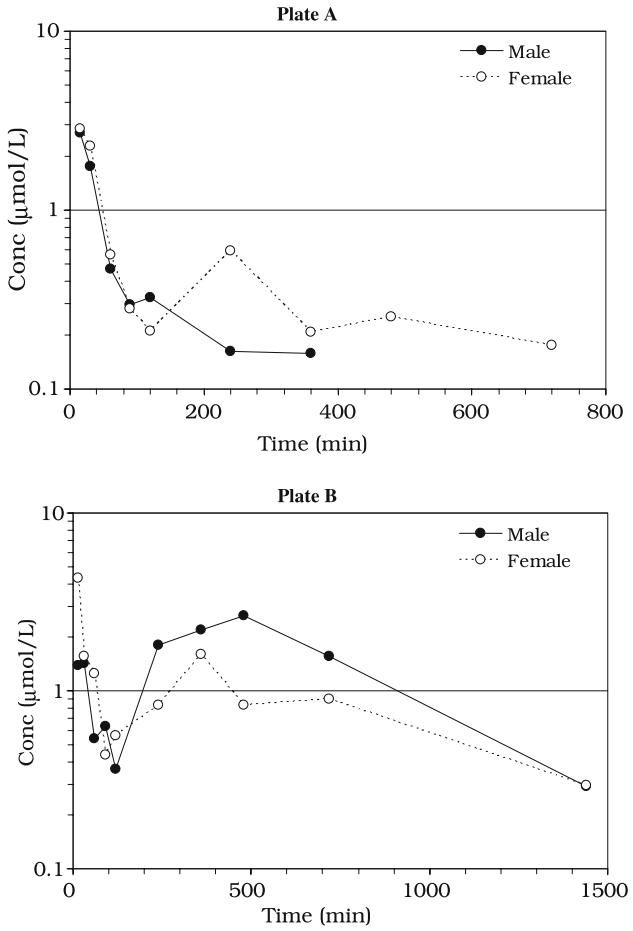

Fig. 3 IUdR plasma concentration time curves for male and female Fischer-344 rats following a single gavage administration (day 1) of IPdR at dosages of 1,200 mg m^{-2} (Plate A) or 6,000 mg m^{-2} (Plate B)

1 IPdR and IUdR C_{max} values indicated that the parent (IPdR) relative to the metabolite (IUdR) peak plasma concentrations were approximately 10–11 times higher for the 1,200 mg m^{-2} group and 26–42 times higher for the 6,000 mg m^{-2}group.

The apparent volume of distribution (*Vz*/*F*), based on the terminal linear phase for each curve (Fig. 3), was 1,780 and 1,570 L m^{-2} for the male and female 1,200 mg m^{-2} groups, and 1,260 and 3,110 L m^{-2} for the male and female 6,000 mg m^{-2} groups, respectively. However, while the *Vz*-*F* for females was slightly larger than males in the 6,000 mg m^{-2} dosage group, lack of bioavailability (*F*) information prevented appropriate comparison of *Vz*/*F* across the two dosage groups. The IUdR half-life values showed a trend to IPdR dose- and sex-dependence. The male and female half-life values were 3.4 and 5.7 h for the 1,200 mg m^{-2} groups and 5.0 and 8.3 h for the 6,000 mg m^{-2} groups, respectively. The apparent IUdR clearance (CL/F) values did not show any sex or IPdR dose dependence but the IUdR CL/F values were much higher than the IPdR CL/F values, based on day 1 results (i.e., 0.433–0.624 L $min^{-1}m^{-2}$). The IUdR AUC values on day 1 were similar for males and females for both IPdR dosage groups. For the males, the increase in AUC was slightly larger than proportional; while for female rats, the increase in AUC between the two IPdR dosage groups approximated a proportional increase.

Day 28 IUdR pharmacokinetic analysis

Using the highest average IUdR concentration for the different groups (Fig. 4), the observed C_{max} values showed no sex dependence and a less than dose proportional increase within sex groups (Tables 3 and 4). When compared to day 1, the C_{max} values for the day 28 groups were much smaller than the day 1 groups for a given IPdR dose and sex. A comparison of the IPdR (parent drug) and IUdR (metabolite) C_{max} values indicates that the parent relative to the metabolite peak plasma concentrations were approximately 12 to 42 times higher in the 1,200 mg m^{-2} day^{-1} groups and 47 to 55 times higher for the 6,000 mg m^{-2} day^{-1} groups. The observed T_{max} values were 15 min for female rats and the male 1,200 mg m^{-2} day^{-1} group, but significantly longer for the male 6,000 mg m^{-2} day^{-1} group.

A comparison of the day 1 and day 28 IUdR apparent volume of distribution (*Vz*/*F*) indicated that the volume of distribution remained relatively unchanged, although lack of information on bioavailability (*F*) again prevented appropriate comparison of *Vz*/*F* between the two dosage groups. The half-life for the terminal linear phase was variable and did not result in any consistent trend in dose or sex. Likewise, a comparison of the day 1 and day 28 half-life values resulted in no clear trend for time, dose or sex. The apparent IUdR clearance values (CL/F) were approximately 2–3 times larger in male rats groups compared to female rat groups for both IPdR dose groups. Additionally, a comparison of the day 1 and day 28 CL/F for IUdR suggested a general trend for a decrease by day 28.

The predicted AUC values were considered reliable for the female 1,200 mg m^{-2} day^{-1} group and the male 6,000 mg m^{-2} day^{-1} group, where less than 27% of the area was extrapolated. However, the predicted IUdR AUC values for the other two dose groups were not reliable as more than 50% of the area was extrapolated. The uncertainty of the AUC values for the male 1,200 mg m^{-2} day^{-1} group and the female 6,000 mg m^{-2} day^{-1} group precluded any further comparison of sex, dose or time.

Days 8 and 15 IUdR pharmacokinetic analysis

The pre-dose concentrations (C_{min}) of IUdR in plasma on both day 8 and 15 were not detectable in the 1,200 mg m^{-2} day^{-1} groups. The mean (± SD) IUdR plasma C_{min}

Table 3 Pharmacokinetic (PK) parameters for IUdR based on noncompartment analysis of concentration time curves for male Fischer-344 rats after a single (day 1) and multiple daily (day 28) Gavage administration(s) of IPdR

PK parameter	Unit	Male rat (1,200 mg m^{-2}, day 1)	Male rat (6,000 mg m^{-2}, day 1)	Male rat (1,200 mg m^{-2}, day 28)	Male rat (6,000 mg m^{-2}, day 28)
Vz/F	L m^{-2}	1,780	1,259	4,295	2,936
$AUC_{(obs)}$	μM min	156.1	1,931	104.2	617.0
$AUC_{(pred)}$	μM min	195.5	2,056	227.5	784.4
AUC_% Extrap_pred	%	20	6	54	21
Half Life	min	201.0	299.1	564.4	266.0
Cl/F	L min^{-1} m^{-2}	6.138	2.918	5.275	7.650
T_{max}	min	15.00	480.0	15.00	240.0
C_{max}	μM	2.693	2.645	0.8401	1.624

Abbreviations used: *Vz/F*, apparent volume of distribution; see Table 1

Table 4 Pharmacokinetic (PK) Parameters for IUdR Based on Noncompartment Analysis of Concentration Time Curves for Female Fischer-344 Rats After a Single (day 1) and Multiple Daily (Day 28) Gavage Administration(s) of IPdR

PK parameter	Unit	Female rat (1200 mg m^{-2}, day 1)	Female rat (6000 mg m^{-2}, day 1)	Female rat (1200 mg m^{-2}, day 28)	Female rat (6000 mg m^{-2}, day 28)
Vz/F	L m^{-2}	1,571	3,106	1,450	6,453
$AUC_{(obs)}$	μM min^{-1}	297.7	1,173	347.2	541.7
$AUC_{(pred)}$	μM min^{-1}	374.6	1,385	477.7	2,183
AUC_%Extrap_pred	%	21	15	27	75
Half life	min	339.9	496.8	400.2	1,627
Cl/F	L min^{-1} m^{-2}	3.203	4.334	2.512	2.749
T_{max}	min	15.00	15.00	15.00	15.00
C_{max}	μM	2.854	4.300	1.960	1.037

Abbreviations used: see Tables 1 and 3

concentrations was 0.188 ± 0.027 μM on day 8 in the 6,000 mg m^{-2} day^{-1} male group compared to 0.433 ± 0.139 μM in the 6,000 mg m^{-2} day^{-1} female group. On day 15, only a single male rat in the 6,000 mg m^{-2} day^{-1} group had a detectable IUdR C_{min} (0.190 μM) while the mean (± SD) IUdR plasma concentration in the female 6,000 mg m^{-2} day^{-1} group was 0.294 ± 0.118 μM.

Discussion

This toxicology and pharmacokinetic study of po IPdR given qd × 28 in Fischer-344 rats was performed to provide additional pre-clinical animal (rodent) data prior to the first clinical phase I trial of po IPdR as a radiosensitizer using a similar dosing schedule. The primary objective was to determine target organ toxicity. Prior pre-clinical studies in other rodents (athymic mice) and non-rodent animals (ferrets) concluded that po IPdR given qd × 6–14 days was well tolerated up to 2 g kg^{-1} day^{-1} with efficient conversion of IPdR to IUdR by hepatic aldehyde oxidase, which resulted in significantly greater in vivo tumor radiosensitization compared to maximum tolerated doses (MTD) of continuous infusion IUdR [8–11, 16]. In addition, significantly less %IUdR-DNA cellular incorporation into normal bone marrow and normal intestinal mucosa were found following po IPdR versus continuous infusion IUdR, suggesting a further improvement in the therapeutic gain for in vivo tumor radiosensitization using po IPdR [8–11, 16]. However, we also found a 30–50% reduction in hepatic aldehyde oxidase activity in cytosol extracts in ferrets following a 14-day IPdR daily dosing schedule with IPdR doses of 1.0 and 1.5 g $kg^{-1}day^{-1}$, suggesting possible enzyme inhibition with repeated daily dosing using this qd × 14 day schedule [10]. Thus, a second objective of this present study was to carefully study plasma pharmacokinetics of IPdR and IUdR throughout the 28-day treatment schedule.

The initial design of this study called for an analysis of plasma samples for IPdR and IUdR by HPLC with UV detection as previously used [8–11, 16]. However, the limit of quantification (LOQ) in plasma for both the parent drug (IPdR) and metabolite (IUdR) was found to be approximately 1 μM (340 ng mL^{-1}). In order to determine if the LOQ would be sufficient to generate a fully defined plasma concentration-time profile for IPdR and IUdR, a simulated kinetic model based on our prior data [10] was created and evaluated. The results of this model indicated that a LOQ of 0.21 μM (70 ng mL^{-1}) would be

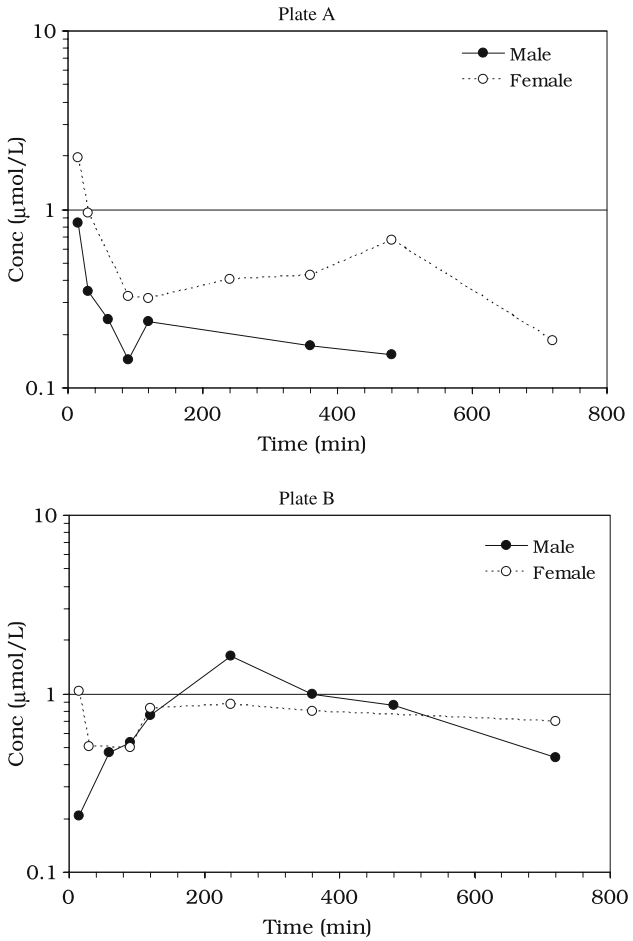

Fig. 4 IUdR plasma concentration time curves for male and female Fischer-344 rats following multiple daily gavage administrations (day 28) of IPdR at dosages of 1,200 mg m^{-2} (Plate A) or 6,000 mg m^{-2} (Plate B)

required to complete the PK arm of this study. As a result of this simulated kinetic modeling, a new method utilizing HPLC coupled with tandem mass spectrometry (MS/MS) as the detection mode was developed and validated for this study. This LC/MS/MS method has a LOQ range of 0.15–5.9 μM (50–2000 mg mL^{-1}) and our results on fully defining the terminal linear phase of IPdR (Figs. 1 and 2) demonstrate that this more sensitive methodology was necessary. This LC/MS/MS method will be used for our clinical phase I study, which illustrates the value of such a kinetic simulation in pre-clinical toxicology and pharmacokinetic study design.

The primary objective of this study was to determine target organ toxicity of po IPdR and its reversibility when administered qd × 28 day. Drug-related effects were clinically evident at 1.0 and 2.0 g kg^{-1} day^{-1} as indicated by observation of hunched posture, rough coat, or thin appearance. The 2.0 g kg^{-1} day^{-1} dosed males experienced significant decreases (8–11%) in group mean body weights beginning on day 5 and continuing through the 28 day treatment period; but the weight loss was readily reversible once treatment was completed. Decreases in some hematological parameters (RBC, Hgb, Hct and total lymphocytes) and generally increased percent reticulocyte counts with some nucleated RBC in peripheral smears were found during treatment (greatest at day 15), with complete reversal by day 42 (i.e., 2 weeks following treatment). No effects on WBC or platelets were found, suggesting that bone marrow toxicity may not be clinically dose limiting using po IPdR in humans. This is in contrast to our prior clinical experience using continuous infusion IUdR for 14 or 28 days [3, 7, 15, 18, 20, 23, 24]. Alterations of several serum chemistry parameters (globulin, albumin, total protein, alkaline phosphatase and alanine aminotransferase) were found in the 1.0 and 2.0 g $kg^{-1}day^{-1}$ treatment groups and were completely reversed within 1–2 weeks after dosing stopped.

No drug-related deaths occurred and only 1 animal (male, 2.0 g kg^{-1} day^{-1} group) had a gross pathology finding, a small thymus gland with microscopic evidence of thymic lymphoid depletion. Drug-related microscopic changes were found in bone marrow (hyperplasia), some lymphoid tissues (lymphoid depletion in thymus as well as in mandibular and mesenteric lymph nodes), liver (increased hepatic cytoplasmic vacuolization), and salivary glands (atrophy of serous and mucinous glands of the mandibular gland). The bone marrow hyperplasia was subtle and felt to represent a response to the anemia and not a direct effect of IPdR. The incidence of lymphoid depletion was dose-dependent and loosely correlated with a slight decrease in circulating lymphocytes. Increased hepatic cytoplasmic vacuolization suggestive of increased glycogen in the cytoplasm was noted in all drug-treated rats at necropsy immediately following treatment (day 29) but not found in the recovery group (day 59) necropsy evaluation. Similar hepatic cytoplasmic changes were noted in ferrets treated with po IPdR up to 1.5 g kg^{-1} day^{-1} × 14 days and sacrificed on day 15 but no observation period to assess reversibility was incorporated into our prior study [10]. The only microscopic finding that persisted at the final autopsy (day 57) was multifocal lobular atrophy in the mandibular salivary glands which was IPdR dose-related although only minimal to mild changes persisted after 4 weeks of observation (day 57). Importantly, no severe histopathologic changes were found at the interim (day 29) or final (day 57) necropsies in any tissue/organ.

As stated previously, the second objective of our study was to determine the pharmacokinetic parameters of IPdR and IUdR following a single gavage (day 1) and after multiple qd administrations (day 28), as well as to measure IPdR/IUdR concentrations prior to dosing on days 8 and 15.

The plasma concentration time curves were analyzed using compartmental modeling for IPdR and non-compartmental analysis for IUdR as described in the ''Materials and methods'' section. IPdR has typical plasma concentration-time curves for oral administration (Figs. 1, 2). For each curve, there is an initial upward phase representing absorption, a well-defined peak plasma concentration, and a terminal linear phase representing elimination. Absorption of po IPdR was not dependent on sex, dose, nor single (day 1) versus multiple administrations (day 28).

Using this sensitive LC/MS/MS methodology, we could accurately measure peak and trough plasma concentrations of IPdR and IUdR. For IPdR, there were no sex-dependent effects on PK parameters although some PK changes were inconsistent (Tables 1, 2). Similar PK inconsistencies were noted with IUdR (Tables 3, 4). However, these more detailed IPdR and IUdR PK data for a 28 day once-daily dosing schedule do not suggest that IPdR reduced the activity of hepatic aldehyde oxidase, the principle enzyme responsible for metabolic clearance of IPdR. This conclusion is in contrast to our prior data in ferrets following a qd $\times$ 14 day IPdR dosing schedule up to 1.5 g kg^{-1} day^{-1} [10].

Based on these additional pre-clinical data, the FDA approved an investigator-initiated IND (#70,333) to allow for initial clinical testing of po IPdR as a radiosensitizer using a once daily $\times$ 28 day schedule with po IPdR dosing beginning 1 week prior to radiation therapy. Using these pharmacokinetic data, our initial pharmacokinetic strategy will involve blood sampling on day 1 at 0, 15, 30, 60, 120, and 240 min and at 24 h (just prior to day 2 po dosing). On days 8, 15 and 22, blood will be sampled at 15, 30, 60, 120, and 240 min. If these rat data are not predictive of po IPdR metabolism in humans, then this initial sampling should point to the best approach. We have already determined in vitro conversion of IPdR to IUdR within 15 min in cytosol extracts of normal human liver [9]. The final pharmacokinetic sampling protocol will monitor IPdR decay in plasma for 2–3 half-lives on days 1, 8 and 22.

Others and we have shown that the % IUdR-DNA incorporation in a cell (in vitro) or tissue (in vivo) correlates directly with the extent of normal cell or tissue cytotoxicity and with the extent of tumor radiosensitization (reviewed in [6] and [26). In prior pre-clinical in vivo testing of po IPdR as a prodrug for IUdR-mediated radiosensitization, we consistently found $\geq$ twofold increases in %IUdR-DNA tumor cell incorporation using four different human tumor xenografts in athymic mice and $\geq$ twofold decreases in %IUdR-DNA incorporation in proliferating dose-limiting normal tissues (bone marrow and intestine) following po IPdR given daily $\times$ 6–14 days compared to MTD doses of continuous intravenous infusions of IUdR for similar time periods [8, 9, 11, 16, 17]. In prior clinical phase I trials of continuous intravenous infusions of IUdR ($\pm$ biochemical modulators), we have measured the %IUdR-DNA incorporation in circulating granulocytes during and follow the IUdR infusion as a surrogate for a proliferating normal tissue (bone marrow) [7, 13, 15, 19]. In general, the %IUdR-DNA incorporation in circulating granulocytes increased linearly with a linear increase in steady-state plasma levels of IUdR with high ($\geq$ 6%) %IUdR-DNA levels in peripheral granulocytes predicting for systemic myelosuppression. Using po IPdR qd $\times$ 14 day in athymic mice, $\leq$ 2% IUdR-DNA incorporation was found in circulating granulocytes [11, 16, 17] and no changes in WBC and platelet counts were found using po IPdR qd $\times$ 14 days in ferrets [10] and using po IPdR qd $\times$ 28 d in rats in this study. Thus, bone marrow toxicity may not be dose limiting for po IPdR given qd in humans at least for 28 days. However, we will closely monitor blood counts weekly during IPdR treatment and for up to 4 weeks following treatment. Additionally, we will measure the %IUdR-DNA incorporation in circulating granulocytes as well as oral mucosa (buccal smear) on a similar time scale during and following IPdR. These in vitro cellular measurements of %IUdR-DNA incorporation in circulating granulocytes and buccal mucosa will be correlated with observed systemic clinical toxicities to bone marrow and the gastrointestinal tract, respectively. Finally, we plan to measure %IUdR-DNA incorporation in tumor on day 8, prior to the initiation of radiation therapy, to assess any tumor selective effect on po IPdR dose escalation during the study, realizing the limitations of a single tumor biopsy as well as intra- and inter-tumor variability. A non-invasive methodology of IUdR tumor ''uptake'' is under development using ^{124}IUdR and positron-emission tomography, which may allow for repeated scanning during IPdR treatment.

References

1. Berry SE, Kinsella TJ (2001) Targeting DNA mismatch repair for human tumor radiosensitization. Semin Radiat Oncol 11:300–315
2. Berry SE, Loh T, Yan T, Kinsella TJ (2003) Role of MutSalpha in the recognition of iododeoxyuridine in DNA. Cancer Res 63:5490–5495
3. Chang AE, Collins JM, Speth PA, Smith R, Rowland JB, Walton L, Begley MG, Glatstein E, Kinsella TJ (1989) A phase I study of intraarterial iododeoxyuridine in patients with colorectal liver metastases. J Clin Oncol 7:662–668
4. Chang CN, Doong SL, Cheng YC (1992) Conversion of 5-iodo-2-pyrimidinone-2′-deoxyribose to 5-iodo-deoxyuridine by aldehyde oxidase. Implication in hepatotropic drug design. Biochem Pharmacol 43:2269–2273
5. Efange SM, Alessi EM, Shih HC, Cheng YC, Bardos TJ (1985) Synthesis and biological activities of 2-pyrimidinone nucleosides. 2. 5-Halo-2-pyrimidinone 2′-deoxyribonucleosides. J Med Chem 28:904–910

6. Kinsella TJ (1996) An approach to the radiosensitization of human tumors. Cancer J Sci Am:184–193
7. Kinsella TJ, Collins J, Rowland J, Klecker R Jr, Wright D, Katz D, Steinberg SM, Glastein E (1988) Pharmacology and phase I/II study of continuous intravenous infusions of iododeoxyuridine and hyperfractionated radiotherapy in patients with glioblastoma multiforme. J Clin Oncol 6:871–879
8. Kinsella TJ, Kunugi KA, Vielhuber KA, McCulloch W, Liu SH, Cheng YC (1994) An in vivo comparison of oral 5-iodo-2′-deoxyuridine and 5-iodo-2-pyrimidinone-2′-deoxyribose toxicity, pharmacokinetics, and DNA incorporation in athymic mouse tissues and the human colon cancer xenograft, HCT-116. Cancer Res 54:2695–700
9. Kinsella TJ, Kunugi KA, Vielhuber KA, Potter DM, Fitzsimmons ME, Collins JM (1998) Preclinical evaluation of 5-iodo-2-pyrimidinone-2′-deoxyribose as a prodrug for 5-iodo-2′-deoxyuridine-mediated radiosensitization in mouse and human tissues. Clin Cancer Res 4:99–109
10. Kinsella TJ, Schupp JE, Davis TW, Berry SE, Hwang HS, Warren K, Balis F, Barnett J, Sands H (2000) Preclinical study of the systemic toxicity and pharmacokinetics of 5-iodo-2-pyrimidinone-2′-deoxyribose as a radiosensitizing prodrug in two, non-rodent animal species: implications for phase I study design. Clin Cancer Res 6:3670–3679
11. Kinsella TJ, Vielhuber KA, Kunugi KA, Schupp J, Davis TW, Sands H (2000) Preclinical toxicity and efficacy study of a 14-day schedule of oral 5-iodo-2-pyrimidinone-2′-deoxyribose as a prodrug for 5-iodo-2′-deoxyuridine radiosensitization in U251 human glioblastoma xenografts. Clin Cancer Res 6:1468–1475
12. Lewandowski GA, Cheng YC (1991) Mechanism and mode of action of 5-iodo-2-pyrimidinone 2′-deoxyribonucleoside, a potent anti-herpes simplex virus compound, in herpes simplex virus-infected cells. Mol Pharmacol 39:27–33
13. McGinn CJ, Kunugi KA, Tutsch KD, Feierabend C, Alberti D, Lindstrom MJ, Wilding G, Arzoomanian RZ, Kinsella TJ (1996) Leucovorin modulation of 5-iododeoxyuridine radiosensitization: a phase I study. Clin Cancer Res 2:1299–1305
14. Rooseboom M, Commandeur JN, Vermeulen NP (2004) Enzyme-catalyzed activation of anticancer prodrugs. Pharmacol Rev 56:53–102
15. Schulz CA, Mehta MP, Badie B, McGinn CJ, Robins HI, Hayes L, Chappell R, Volkman J, Binger K, Arzoomanian R, Simon K, Alberti D, Feierabend C, Tutsch KD, Kunugi KA, Wilding G, Kinsella TJ (2004) Continuous 28-day iododeoxyuridine infusion and hyperfractionated accelerated radiotherapy for malignant glioma: a phase I clinical study. Int J Radiat Oncol Biol Phys 59:1107–1115
16. Seo Y, Yan T, Schupp JE, Colussi V, Taylor KL, Kinsella TJ (2004) Differential radiosensitization in DNA mismatch repair-proficient and -deficient human colon cancer xenografts with 5-iodo-2-pyrimidinone-2′-deoxyribose. Clin Cancer Res 10:7520–7528
17. Seo Y, Yan T, Schupp JE Radivoyevitch T, Kinsella TJ (2005) Schedule-dependent drug effects of oral 5-iodo-2-pyrimidinone-2′-deoxyribose as an in vivo radiosensitizer in U251 human glioblastoma xenografts. Clin Cancer Res 11:7499–7505
18. Sondak VK, Robertson JM, Sussman JJ, Saran PA, Chang AE, Lawrence TS (1998) Preoperative idoxuridine and radiation for large soft tissue sarcomas: clinical results with 5 year follow-up. Ann Surg Oncol 5:106–112
19. Speth PA, Kinsella TJ, Belanger K, Klecker RW Jr, Smith R, Rowland JB, Collins JM (1988) Fluorodeoxyuridine modulation of the incorporation of iododeoxyuridine into DNA of granulocytes: a phase I and clinical pharmacological study. Cancer Res 48:2933–2937
20. Sullivan FJ, Herscher LL, Cook JA, Smith J, Steinberg SM, Epstein AH, Oldfield EH, Goffman TE, Kinsella TJ, Mitchell JB, Glatstein E (1994) National Cancer Institute (phase II) study of high-grade glioma treated with accelerated hyperfractionated radiation and iododeoxyuridine: results in anaplastic astrocytoma. Int J Radiat Oncol Biol Phys 30:583–590
21. Taverna P, Hwang HS, Schupp JE, Radivoyevitch T, Session NN, Reddy G, Zarling DA, Kinsella TJ (2003) Inhibition of base excision repair potentiates iododeoxyuridine-induced cytotoxicity and radiosensitization. Cancer Res 63:838–846
22. Turner DP, Cortellino S, Schupp JE, Caretti E, Loh T, Kinsella TJ, Bellacosa A (2006) The DNA N-glycosylase MED1 exhibits preference for halogenated pyrimidines and is involved in the cytotoxicity of 5-iododeoxyuridine. Cancer Res 66:7686–7693
23. Urtasun RC, Cosmatos D, DelRowe J, Kinsella TJ, Lester S, Wasserman T, Fulton DS (1993) Iododeoxyuridine (IUdR) combined with radiation in the treatment of malignant glioma: a comparison of short versus long intravenous dose schedules (RTOG 86–12). Int J Radiat Oncol Biol Phys 27:207–214
24. Urtasun RC, Kinsella TJ, Farnan N, DelRowe JD, Lester SG, Fulton DS (1996) Survival improvement in anaplastic astrocytoma, combining external radiation with halogenated pyrimidines: final report of RTOG 86–12, phase I–II study. [see comment]. Int J Radiat Oncol Biol Phys 36:1163–1167
25. Yan T, Seo Y, Schupp JE, Zeng X, Desai AB, Kinsella TJ (2006) Methoxyamine potentiates iododeoxyuridine-induced radiosensitization by altering cell cycle kinetics and enhancing senescence. Mol Cancer Ther 5:893–902
26. Zarling DAV AK, Kinsella T (2004) New radiosensitizing (RS) regimens, drugs, prodrugs and candidates: Capecitabine, Gemcitabine, Fludarabine, IPdR, Avastin, Veglin, Gleevac, Radvac, Erbitux or Irressa. Clin Adv Hematol Oncol 2:793–805