Cancer Chemotherapy and Pharmacology (2019) 84:93–103
https://doi.org/10.1007/s00280-019-03851-0

ORIGINAL ARTICLE

A single-center, open-label study investigating the excretion balance, pharmacokinetics, metabolism, and absolute bioavailability of a single oral dose of [^{14}C]-labeled idasanutlin and an intravenous tracer dose of [^{13}C]-labeled idasanutlin in a single cohort of patients with solid tumors

Zsuzsanna Pápai[1] · Lin-Chi Chen[2] · Daniel Da Costa[2] · Steven Blotner[2] · Faye Vazvaei[2] · Michelle Gleave[3] · Russell Jones[4] · Jianguo Zhi[2]

Received: 8 November 2018 / Accepted: 25 April 2019 / Published online: 6 May 2019

Abstract

Purpose Idasanutlin, a selective small-molecule MDM2 antagonist in phase 3 testing for refractory/relapsed AML, is a non-genotoxic p53 activator with oral administration. To determine the need to conduct dedicated trial(s) for organ impairment on pharmacokinetic (PK) exposure and/or drug–drug interactions, a single dose of [^{14}C]- and [^{13}C]-labeled idasanutlin was evaluated.

Methods This study was an open-label, non-randomized, single-center trial of idasanutlin to investigate the excretion balance, pharmacokinetics, metabolism, and absolute bioavailability of a single oral dose of [^{14}C]-labeled idasanutlin and an IV tracer dose of [^{13}C]-labeled idasanutlin in a single cohort of patients with solid tumors. After completing cycle 1 assessments, patients could have participated in an optional treatment extension of idasanutlin. Clinical endpoints were PK, and safety/tolerability.

Results Co-administration of an oral dose of idasanutlin with an IV tracer dose revealed low systemic CL, a moderate V_d, and a moderate (40.1%) absolute bioavailability of idasanutlin. Idasanutlin and its major inactive metabolite, M4, were the major circulating moieties in plasma, and excretion of idasanutlin-associated radioactivity was primarily via the fecal route (91.5% of the dose), with negligible amounts recovered in urine, following oral administration.

Conclusion The clinical implications of this study support the conclusion that renal impairment is unlikely to significantly impact exposure to idasanutlin and M4 metabolite, whereas a significant hepatic impairment may potentially alter exposure to the parent drug and/or metabolite(s). The potential for drug–drug interactions is low.

Keywords Idasanutlin · MDM2 antagonist · Mass balance · Absolute bioavailability · Metabolic profiling

Introduction

Cancer remains a major cause of morbidity and mortality worldwide, despite recent successes with drugs providing survival benefit to patients. There remains a high unmet medical need for new, effective, and safe treatments that can be used in all phases of cancer treatment.

The tumor suppressor gene encoded by the TP53 gene (p53) plays a pivotal role in protection from cancer development. It is a transcription factor that is activated following cellular stress and regulates multiple downstream genes implicated in cell cycle control, apoptosis, DNA repair, and senescence. In non-stressed cells, the level of p53 is

✉ Jianguo Zhi
jianguo.zhi8@gmail.com

[1] PRA Magyarország Kft, Fázis I-es Klinikai Farmakológiai Vizsgálóhely, Budapest, Hungary

[2] Roche Innovation Center of New York, New York, NY, USA

[3] York Bioanalytical Solutions, Sandwich, UK

[4] Roche Innovation Center of Basel, Basel, Switzerland

controlled tightly by Murine Double Minute-2 (MDM2). However, in cancer cells overexpressing MDM2, this feedback loop is dysregulated. Stress-induced p53 activation mechanisms in these tumors are believed to be inadequate, leading to inefficient cell growth arrest and/or apoptosis. Therefore, blocking the p53–MDM2 interaction is expected to overcome the oncogenic consequences of MDM2 overproduction and to restore p53 function. Treatment of cancer cells expressing functional p53 with small-molecule MDM2 antagonists resulted in the concurrent transcriptional activation of p53 downstream genes, cell cycle arrest, and apoptosis [1].

Idasanutlin (RO5503781, RG3788) is a potent and selective inhibitor of the p53–MDM2 interaction [2]; it is currently in phase 3 development (NCT02545283) for relapsed/refractory acute myeloid leukemia (AML). It binds on the surface of MDM2 at the p53 binding pocket and mimics the interaction of three critical amino acids from p53, thus preventing the p53–MDM2 protein–protein interaction. Treatment of cultured human cancer cells harboring functional p53 with idasanutlin leads to stabilization and accumulation of p53 protein, blocks cell cycle progression in G1 and G2 phases, and induces apoptosis.

Following scheduling optimization and translational efforts [3, 4] and three phase 1 studies [5–7] including drug–drug interaction, formulation optimization, and food-effect assessments, the present study was conducted to (1) characterize the mass balance in excreta, metabolism routes, and rates of elimination of a single dose of [^{14}C]-labeled idasanutlin, (2) determine plasma concentrations, total drug-related radioactivity, and related pharmacokinetic (PK) parameters following oral administration of idasanutlin, as well as (3) characterize intravenous (IV) pharmacokinetics and determine absolute oral bioavailability of idasanutlin using a tracer dose of [^{13}C]-labeled idasanutlin. To provide potential medical benefits with minimal toxicity to the participating patients, the doses given in all 28-day cycles were kept at 1000–1200 mg per cycle, which were 20% less than the maximum tolerated dose found in patients with solid tumors [5]: three 400-mg single doses administered in 10 days apart in the first assessment cycle, followed by 200 mg QD × 5 days in subsequent 28-day treatment cycles.

Materials and methods

Overall study design

This study was an open-label, non-randomized, phase I study of idasanutlin in a single cohort of patients with solid tumors. In the first period, the disposition, metabolic profile, and the rate and routes of elimination of the study drug were characterized using [^{14}C]-labeled 100 mg containing 100 µCi in MBP (microprecipitated bulk powder) formulation administered with unlabeled (300 mg in SDP, spray-dried powder that is a phase 3 trial and to-be-marketed formulation and dosage strength) oral idasanutlin (patients had the liberty taking the drug with or without a meal), followed by an intravenous administration using a [^{13}C]-labeled isotopic idasanutlin at oral peak time [4], i.e., 6-h post oral administration to allow the quantification of absolute oral bioavailability. Following the mass balance and absolute bioavailability portion of the study, idasanutlin was administered to patients in the form of an oral aqueous dispersion to assess its palatability. A third oral dose of idasanutlin was then administered to patients to complete the first 28-day treatment cycle. An overview of the study design is presented in Fig. 1.

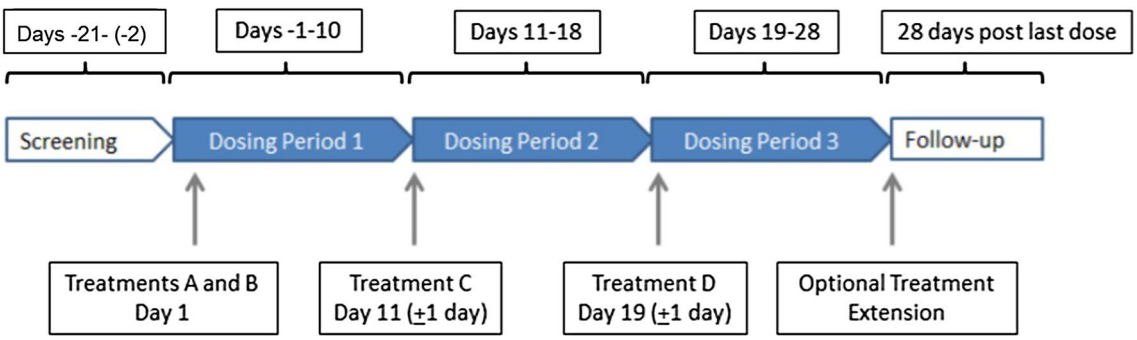

Fig. 1 Overall study design. Treatment A: Single dose of 400 mg oral idasanutlin (two 50 mg containing 100 µCi [^{14}C]-idasanutlin microprecipitated bulk powder [MBP] hard gelatin capsules and one 300 mg idasanutlin spray-dried powder [SDP] tablet). Treatment B: Single dose of 100 µg intravenous [^{13}C]-idasanutlin administered as a 15-min infusion 5 h 45 min after Treatment A. Treatment C: Single dose of placebo dispersed in 10 mL of drinking water, followed 1 h later by 400 mg oral idasanutlin SDP tablet dispersed in 10 mL of drinking water. Treatment D: Single dose of 400 mg oral idasanutlin SDP tablet. Optional Treatment Extension: Patients who completed Treatment Cycle 1 were given an option to enter the Optional Treatment Extension between days 29 and 42. Up to 200 mg oral idasanutlin (SDP) once daily × 5 days, followed by 23 days of rest per 28-day cycle

The [^{14}C]-radioactive dose of a maximum 3.7 MBq (100 μCi) had been chosen based on calculations using the tissue distribution data from studies in rats [2] to identify the critical organs. In addition, based on the potential variability of up to 10% (impurity) in idasanutlin content due to technical reasons, a dose of radioactivity of 3.7 MBq (100 μCi) was expected to provide sufficient exposure to meet the objectives of the study, while limiting the radioactive burden to the patients. The effective radiation burden in this study from [^{14}C]-labeled idasanutlin was estimated to be 0.06 mSv, below 0.1 mSv to be of trivial risk in healthy male subjects [8].

During the Treatment Cycle, safety assessments were performed and blood samples for laboratory safety tests and PK measurements were collected. Patients who completed the Treatment Cycle had the option of entering the Optional Treatment Extension part of the study to continue treatment with idasanutlin if, in the opinion of the investigator, it was felt that the patient could benefit from continued treatment. Treatment continued with idasanutlin SDP tablets at a dose of up to 200 mg once daily (QD) for 5 days, followed by 23 days of rest per 28-day cycle, until the development of progressive disease, unacceptable toxicity, consent withdrawal, or any other criteria for removal.

Ethics and study conduct

The study was conducted in accordance with the principles of Good Clinical Practice. Independent Ethics Committee approval was obtained from the participating site.

Selection of study population

Approximately 8–10 male or female adult patients (≥ 18 years of age) with solid tumors were planned to be enrolled in the study to achieve six evaluable patients. The study population comprised of adult, treatment-refractory patients with solid tumors including lymphoma and with adequate bone marrow, hepatic, renal, and heart functions (ClinicalTrials.gov Identifier: NCT02828930).

Concomitant medications and dietary restrictions

Idasanutlin metabolism is mainly mediated by CYP3A, CYP2C8, and UGTs [4], while it is a CYP2C8 inhibitor and M4 metabolite is an OATP1B1/3 inhibitor [unpublished]. Hence, CYP2C8 inhibitors, substrates or inducers, OATP1B1/3 substrates, or strong CYP3A4 inducers were to be avoided for patient safety or potential for diminished activity. Substrates and inhibitors had to be discontinued 7 days, while inducers had to be discontinued 14 days, prior to start of study medication.

Assessments

Blood, urine, feces (and toilet tissue), and vomitus (as appropriate) samples were collected for PK, radioactivity, and metabolic profiling assessments. The collection of blood, urine, feces and vomitus samples (as appropriate) for radioactivity counting began prior to administration of the radioactive doses and continued until at least one of the following two recovery criteria are met: cumulative recovery of radioactivity exceeds 90% of the administered dose or radioactivity in urine and feces (and toilet tissues) was less than 1% of the administered dose over a 24-h period on two consecutive PK sample collection days, as determined by quick liquid scintillation counts. Blood samples were taken for the following PK analyses: concentration of idasanutlin, its metabolites, and the concentration of [^{13}C]-idasanutlin in plasma, analysis of total [^{14}C]-idasanutlin radioactivity and quick counts in whole blood and plasma, and metabolic profiling in plasma. Urine (pre-dose, 0–6 h, 6–12 h, 12–24 h followed by daily) and fecal (daily) samples were taken for analysis of total [^{14}C]-idasanutlin radioactivity and quick counts, metabolite profiling (if there were significant levels of radioactivity present), and concentration of idasanutlin (if measurable). Vomitus sample, collected if a patient vomited within the time period from dose administration to 4 h after oral dose, was included in the total radioactivity measurements and in the determination of the mass balance, as appropriate, but was not used for metabolite profiling.

Blood samples for plasma concentrations of idasanutlin (with its inactive M4 metabolite RO6802287) with K3EDTA as anticoagulant and ^{14}C/^{13}C levels were collected at baseline (control) and periodic post-dose time points following drug administration on assessment days. Plasma PK samples were analyzed for idasanutlin and its M4 metabolite by a validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) method at Q^2 Solutions (formerly Quintiles), Ithaca, NY, USA. The stable isotope [^{13}C]-labeled idasanutlin was measured in plasma by a specific LC/MS–MS method. Total ^{14}C radioactive counting was determined in whole blood, plasma, urine, feces, and vomit (as appropriate) by liquid scintillation counting at PRA Health Sciences—Early Development Services, Bioanalytical Laboratory, Amerikaweg 18, 9407 TK, Assen, The Netherlands.

Metabolic profiling was investigated with ^{14}C-labeled idasanutlin and performed at Unilabs—York Bioanalytical Solutions, Discovery Park, Ramsgate Road, Sandwich, Kent CT13 9ND, United Kingdom. Metabolic profiling was intended to be conducted in plasma, urine, and fecal samples and was assessed if there were significant levels of radioactivity present. Plasma samples were pooled across seven subjects and metabolic profiles were acquired for the 6-, 10-, 24-, 48- and 96-h time points. Profiles were also acquired from each individual 24-h plasma sample. Samples from

the patient who dropped out were excluded from metabolite identification pools due to the low recovery of dose (79.4%), atypical PK profile and failure to complete the study, but were investigated separately.

Feces were pooled across subjects and time points up to 264 h (representing 87.0% of the dose). Metabolite profiles in urine were not investigated, since negligible amounts of the dose (< 0.1%) were excreted on average via this route.

The extraction of drug-related material from human plasma and feces by protein precipitation was considered to be essentially complete with recovery of all radioactivity (via liquid scintillation counting) in each sample (mean = 91.2% and 94.5%, respectively). Plasma and fecal extracts were analyzed by HPLC combined with mass spectrometric and radiometric detection. Radiometric detection was achieved following fractionation of the post column eluent into 96-well Scintplates, enabling solid scintillation counting using a Microbeta instrument. Identification and structural elucidation of metabolites was conducted by HPLC-SSC combined with high-resolution accurate mass spectrometry (HRMS). Metabolite identification was achieved following correlation of retention times and product ion spectra with that of reference compounds where available.

Palatability and aftertaste of an aqueous dispersion of idasanutlin were assessed via a questionnaire provided in the protocol. Palatability and aftertaste were also assessed for an unblinded placebo administered 1 h prior to the idasanutlin aqueous dispersion. The duration of aftertaste was also recorded during 3 h after drug administration.

Tumor response was not a primary endpoint but was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 or Cheson criteria for NHL. Patient safety was evaluated on the basis of adverse events (AEs), laboratory abnormalities (through weekly monitoring of hematological changes), vital signs, electrocardiogram (ECG) assessments, physical examinations, and Eastern Cooperative Oncology Group (ECOG) performance status.

Planned sample size and data analysis

Because this was a phase 1 mass balance study, no formal statistical hypothesis testing was conducted; nor formal sample size calculation was performed. The sample size of six evaluable patients was conventional for human mass-balance studies.

Following oral administration of idasanutlin, the following key PK parameters of unchanged idasanutlin and M4 were planned to be included: t_{max}, C_{max}, AUC, $t_{1/2}$, CL/F, CL_r, V_d/F, amount excreted (cumulative) in urine, amount excreted (cumulative) in feces, metabolite–parent ratios (based on AUC or amount, when appropriate), where the contribution from the IV tracer dose (100 µg) was presumed to be negligible (< 0.2%) because oral doses (100 mg MBP + 300 mg SDP) were several thousand-fold higher.

Following the IV administration of [^{13}C]-labeled idasanutlin, the following key PK parameters were planned to be included: t_{max}, C_{max}, AUC, $t_{1/2}$, absolute bioavailability F (ratio of dose normalized PO $AUC_{0-\infty}$/IV $AUC_{0-\infty}$), CL_r, and V_d. Absolute bioavailability (F) = AUC_{oral}/AUC_{IV}, adjusted for dose difference, where for AUC_{oral}, unchanged idasanutlin was used, and for AUC_{IV}, idasanutlin as measured from [^{13}C]-labeled idasanutlin was used. Since the oral administration used two formulations (100 mg MBP and 300 mg SDP), further dose adjustment in absolute bioavailability calculation was made to count the apparent difference in relative bioavailability. Thus, the total dose of oral idasanutlin was 760 mg (in terms of MBP formulation) specified by counting 2.2-fold higher relative bioavailability of SDP to MBP [6] from 400 mg (in terms of mixed MBP and SDP formulations) administered in Treatment A.

Following oral administration of [^{14}C]-labeled idasanutlin, the following key PK parameters were planned to be included: t_{max} (whole blood and plasma), C_{max} (whole blood and plasma), AUC (whole blood and plasma), cumulative amount of radioactivity excreted in urine as percentage of dosed radioactivity $A_{e,\,ur}$ (%), cumulative amount of radioactivity excreted in feces as percentage of dosed radioactivity $A_{e,\,fec}$ (%), cumulative amount of radioactivity excreted in feces on toilet tissue as a percentage of dosed radioactivity $A_{e,\,toilet\ tissue}$ (%), and cumulative amount of radioactivity in vomit as percentage of dosed radioactivity $A_{e,\,vom}$ (%) (as appropriate). Mass balance = cumulative radioactivity recovery in urine and feces.

The PK parameters were derived using Phoenix WinNonlin v6.2/PKS v4.02 software (Certara, Princeton, NJ) that included non-compartmental analysis, plotting and tabulating, and descriptive statistics, wherever appropriate.

Results

Demographics and baseline characteristics

In total, 8 patients were enrolled in the study. All 8 enrolled patients were white; none of these patients were of Hispanic or Latino origin. Of the 8 patients, 5 patients (62.5%) were females. The median age of these patients at the time of screening was 46.5 years (range 33–73 years). The median body mass index at baseline was 27.1 kg/m^2 (range 20.8–31.2 kg/m^2).

All 8 patients enrolled in the study had confirmed advanced malignancies. Two patients (25%) had rectal cancer, and one patient each (12.5%) had head and neck cancer, bile duct cancer, breast cancer, melanoma, soft tissue sarcoma, and uterine cancer.

Pharmacokinetics

Excretion balance (Treatment A)

No patient vomited during the assessment period; therefore, no vomit sample was collected and analyzed. The mean cumulative recovery of total radioactivity in feces ($A_{e,\,fec}$ [%]) versus time following a single oral dose administration of 100 µCi [^{14}C]-idasanutlin is shown in Fig. 2a. Near complete recovery of administered radioactivity (mean of 91.6%) was achieved between 24 and 264 h post-dose for all eight patients (Table 1). One patient (Subject 3) was hospitalized with Grade 3 sepsis on Study Day 7 and hence had no more samples since, which partially contributed to the relatively low (79.2%) recovery. The cumulative recovery of total radioactivity in urine ($A_{e,\,ur}$ [%]) was close to zero, which suggests that the fecal route is the main route of elimination and the total excretion is similar to fecal excretion.

Blood-to-plasma partitioning (Treatment A)

Total radioactivity in plasma and whole blood mean concentration–time profiles following administration of a single oral dose of 100 µCi [^{14}C]-idasanutlin are presented in Fig. 2b with PK parameters summarized in Table 2. In the patient (Subject 3) who dropped out, both blood and plasma

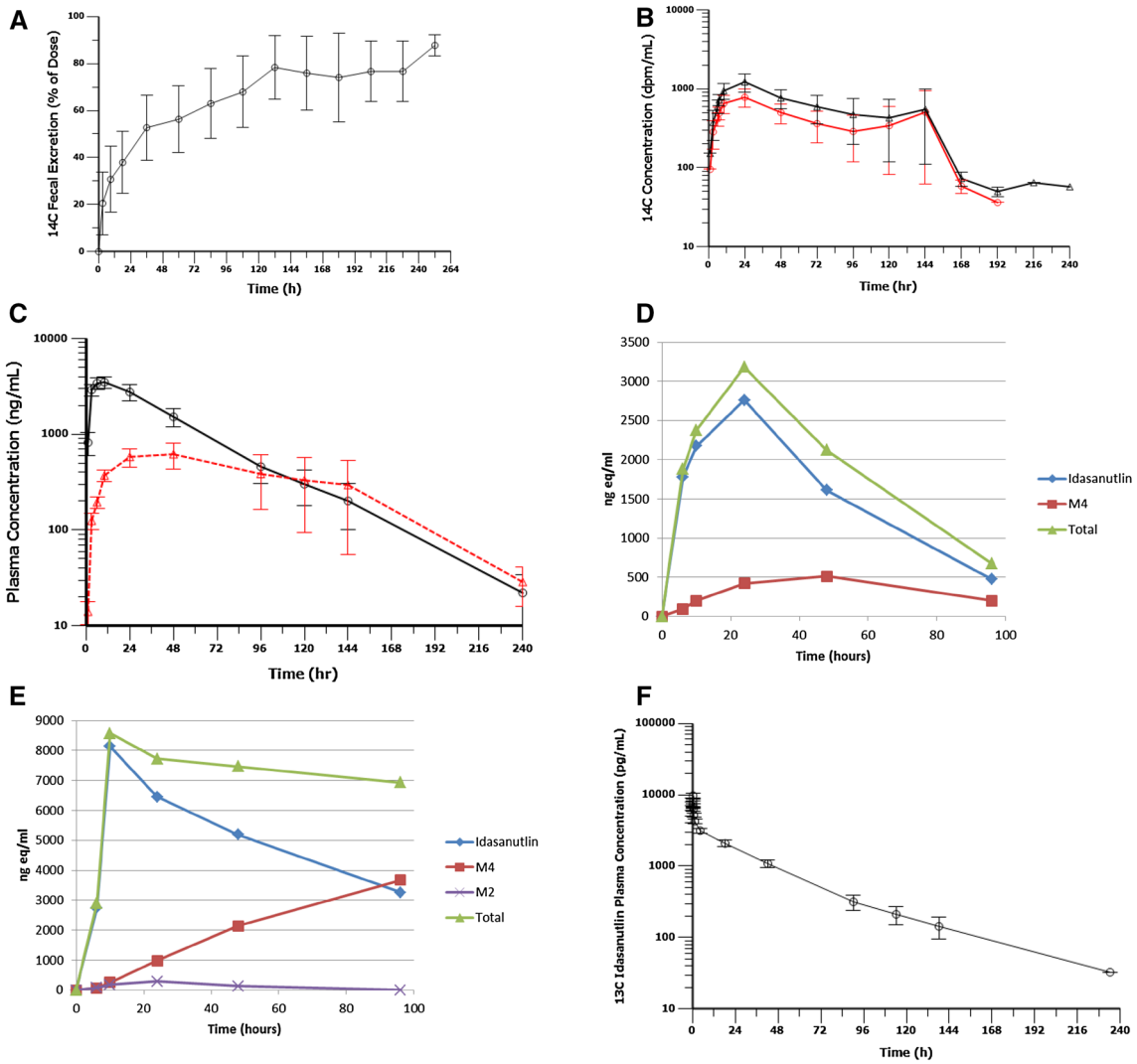

Fig. 2 Mean (±SE) cumulative percent of [^{14}C]-idasanutlin radioactivity recovery in feces (**a**), [^{14}C]-idasanutlin radioactivity concentration (semi-log scale) versus time profiles in plasma (black) and in whole blood (red) (**b**), idasanutlin (black) and M4 metabolite (red) concentration (semi-log scale) versus time profiles in plasma (**c**), plasma time profile of pool 7 subjects (**d**) and Subject 3 only (**e**) for total radioactivity, idasanutlin and metabolite M4 following a single oral dose of 100 µCi [^{14}C]-idasanutlin in 760 mg MBP Eq. idasanutlin, as well as mean (±SE) idasanutlin concentration (semi-log scale) versus time profiles in plasma following a single intravenous dose of 100 µg [^{13}C]-idasanutlin (**f**)

Table 1 Summary of excretion balance for idasanutlin [^{14}C]-radioactivity in excreta following a single oral dose of 100 µCi [^{14}C]-idasanutlin in all 8 patients

Subj	Excretion balance (% of dose)			
ID	Urine	Feces	Toilet tissue	Total
1	0.0	93.7	0.0	93.7
2	0.0	98.6	0.3	98.9
3	0.2	79.2	0	79.4
4	0.0	97.0	0.1	97.1
5	0.0	83.3	0.0	83.3
6	0.0	106.2	0.2	106.4
7	0.0	77.7	0.2	77.9
8	0.1	96.4	0.0	96.5
Mean	0.0	91.5	0.1	91.7
SD	0.1	10.2	0.1	10.3
CV (%)	198	11	120	11

radioactivity did not have substantial decline (estimated $t_{1/2}$ values would approximate 710 h and 277 h for blood and plasma, respectively, leading to inaccurate determination of $AUC_{0-\infty}$) at the time of hospitalization on Study Day 7, contributing to large inter-patient variabilities ($\geq 55\%$) in the two AUC_{0-last} parameters. In addition, lack of Subject 3 data post day 7 (144 h) contributed unusual pattern of Fig. 2b, c ($N=7$ versus $N=8$ in earlier time points).

The mean blood–plasma ratio for total radioactivity was the same at 65% for C_{max} and AUC_{0-last} with $\leq 11\%$ inter-patient variability, indicating no or little blood cell penetration of idasanutlin, by taking hematocrit dilutional effect in the blood into consideration.

Oral pharmacokinetics of idasanutlin and M4 metabolite (Treatment A)

Due to trivial amount of radioactivity, idasanutlin and M4 metabolites in urine samples were not measured. As a result, PK parameter CL_r was not able to be quantified and was assumed to be 0. The fecal contents of idasanutlin and M4 or other metabolites are best estimated from metabolic profiling results below.

Mean plasma concentration–time profiles for idasanutlin and M4 metabolite are presented in Fig. 2c with PK parameters summarized in Table 3. AUC_{0-last} was on average $>90\%$ of $AUC_{0-\infty}$. While the median t_{max} for idasanutlin in plasma was 8 h, it took 24 h for M4 metabolite to reach its peak concentration. The mean $t_{1/2}$ values were over 1 day and over 2 days (excluding the patient who dropped out) for idasanutlin and M4 metabolite, respectively. The mean M4 metabolite–parent (M/P) ratios for C_{max} and AUC_{0-last} were 15.8% and 29.0%, respectively, following the single oral dose of idasanutlin. The patient, who dropped out and would exhibit a much longer $t_{1/2}$, had no substantial elimination for M4 metabolite even after 7 days, which made $t_{1/2}$ and $AUC_{0-\infty}$ to be indeterminate and explained the unusual high radioactivity counts in plasma and blood in this patient, affecting mean profiles in Fig. 2b, c. Note that this patient's idasanutlin pharmacokinetics was much more benign than M4 metabolite, which should have less impact on absolute bioavailability estimation for the patient.

Plasma balance (Treatment A)

Combining plasma PK exposure parameters allows for calculation of the plasma balance, which is presented in Fig. 2d (7 patients pool) and Fig. 2e (Subject 3 only) with parameters summarized in Table 2. Plasma radioactivity peak concentrations and AUC_{0-last} were similar to the sum of idasanutlin and M4 metabolite for C_{max} and AUC_{0-last} parameters, suggesting that both species are the main circulating moieties in plasma without the likelihood of other circulating major metabolites.

Tracer-dose intravenous pharmacokinetics and absolute bioavailability (Treatment B)

Mean idasanutlin concentration versus time profiles following a single IV dose of 100 µg [^{13}C]-idasanutlin is presented in Fig. 2f with PK parameters summarized in Table 3. Administration of the IV tracer dose at 5.75 h following the oral administration resulted in a median t_{max} for [^{13}C]-idasanutlin of 0.25 h, which coincided with the end of the 15-min infusion time. Thereafter, plasma concentrations of idasanutlin declined in a bi-exponential fashion, with a mean terminal $t_{1/2}$ value of approximately 1 day, similar to oral administration of idasanutlin. Interestingly, the PK characteristics following IV administration for the patient (Subject 3) who dropped out were within the range of PK characteristics for the other 7 patients, indicating no relation between PK characteristics and this patient's disease status. The interpatient variability with the clearance value (Table 3) for IV administration (CV 28%) was approximately half of the variability for oral administration (CV 54%).

In comparison with the IV PK exposure, the absolute bioavailability of the oral dosing was estimated. The mean exposure ratio (oral/IV) for idasanutlin dose-normalized $AUC_{0-\infty}$, as the measure of absolute bioavailability, was 18.2% and 40.1% for MBP and SDP formulations, respectively.

Metabolite profiling (Treatment A)

In the pooled plasma samples after a single oral dose, irrespective of the sampling time (6–96 h), parent compound (idasanutlin) was the major drug-related component

Table 2 Summary of idasanutlin radioactivity PK parameters following a single oral dose of 100 µCi ^{14}C idasanutlin

Matrix	^{14}C in whole blood				^{14}C in plasma				Blood/plasma		Conversion	^{14}C in plasma		Σ = M4 + idasanutlin[a]		Σ/^{14}C	
Subj	$t_{½}$	t_{max}	C_{max}	AUC_{last}	$t_{½}$	t_{max}	C_{max}	AUC_{last}	C_{max}	AUC_{last}	Factor	C_{max}	AUC_{last}	C_{max}	AUC_{last}	C_{max}	AUC_{last}
No.	h	h	dpm/mL	dpm·h/mL	h	h	dpm/mL	dpm·h/mL	%	%	ng Eq/dpm	ng-Eq/mL	ng-Eq·h/mL	ng/mL	ng h/mL	%	%
1	28.9	24	1590	81,249	30	24	2693	134,216	59	61	3.320	8941	445,598	3790	224,397	42	50
2	44.6	10	428	41,824	46.2	24.03	686	59,509	62	70	3.714	2548	221,021	3915	264,271	154	120
3		24	1689	206,715		24	2363	286,448	71	72	3.517	8311	1,007,471	7920	639,727	95	63
4	24.7	3	742	18,812	26	3	1135	25,997	65	72	3.439	3903	89,396	5131	142,129	131	159
5	19.2	31.25	807	35,225	15.1	31.25	1429	62,347	56	56	3.417	4882	213,014	3004	133,586	62	63
6	33.2	10	702	40,744	32.4	10	1056	61,871	66	66	3.349	3536	207,177	4458	275,861	126	133
7	22.1	6	818	38,125	22	6	1150	57,880	71	66	3.304	3800	191,250	6830	281,982	180	147
8	58.4	7	275	16,193	93.1	7	398	29,476	69	55	3.263	1299	96,172	1983	86,749	153	90
N	7	8	8	8	7	8	8	8	8	8	8	8	8	8	8	8	8
Mean	33		881	59,861	38		1364	89,718	65	65	3.415	4652	308,887	4629	256,088	118	103
SD	14		506	62,548	26		789	86,043	6	7	0.146	2674	302,662	1957	171,540	48	42
Median		10				17											
CV %	42		57	105	69		58	96	8	11	4	58	96	42	67	41	41

[a]Derived from data in Table 3

Table 3 Summary of PK parameters and absolute bioavailability following a single oral and IV dose of idasanutlin

Route	po 760 mg idasanutlin MBP Eq.												
Analyte	Idasanutlin (I)							M4 metabolite				Ratio of M4/I	
Subj	$t_{½}$	t_{max}	C_{max}	AUC_{last}	$AUC_{0-\infty}$	V_d/F	CL/F	$t_{½}$	t_{max}	C_{max}	AUC_{last}	C_{max}	AUC_{last}
No.	h	h	ng/mL	ng·h/mL	ng·h/mL	L	L/h	h	h	ng/mL	ng·h/mL	%	%
1	26.2	10	3320	190,503	190,703	79.2	2.1	29	24	470	33,894	14.2	17.8
2	43.1	3	3350	191,456	194,258	127.9	2.06	53	24	565	72,815	16.9	38.0
3	67.7	10	5940	409,545	494,315	79.0	0.81		120	1980	230,182	33.3	56.2
4	20.8	3	4750	117,207	118,080	101.7	3.39	25	10	381	24,922	8.0	21.3
5	15.0	10	2600	104,352	104,583	82.6	3.82	16	48	404	29,234	15.5	28.0
6	31.4	10	3920	229,718	230,432	78.5	1.74	32	24	538	46,143	13.7	20.1
7	20.3	6	5790	210,401	211,674	55.4	1.89	22	24	1040	71,581	18.0	34.0
8	25.4	6	1850	74,546	76,068	192.4	5.26	188	24	133	12,203	7.2	16.4
N	8	8	8	8	8	8	8	7	8	8	8	8	8
Mean	31.2		3940	190,966	202,514	99.6	2.63	52		689	65,122	15.8	29.0
SD	17.0		1463	104,302	130,344	43.0	1.42	61		581	70,087	8.1	13.5
Median		8							24				
CV %	54		37	55	64	43	54	117		84	108	51	47

Route	IV 100 µg idasanutlin							Absolute	
Analyte	Idasanutlin						760 mg Eq.	Bioavailability	
Subj	$t_{½}$	C_{max}	AUC_{last}	$AUC_{0-\infty}$	V_d	CL_t	$AUC_{0-\infty}$	MBP	SDP
No.	h	pg/mL	pg·h/mL	pg·h/mL	L	L/h	ng·h/mL	%	%
1	24.5	9650	117,027	120,602	29.4	0.83	916,572	20.8	45.8
2	42.8	8580	145,237	147,254	41.9	0.68	1119,133	17.4	38.2
3	40.8	8310	186,131	208,715	28.2	0.48	1,586,236	31.2	68.6
4	21.0	9680	118,608	119,575	25.3	0.84	908,773	13.0	28.6
5	15.2	10,900	99,654	100,963	21.7	0.99	767,318	13.6	30.0
6	33.0	8920	185,527	195,198	24.4	0.51	1,483,506	15.5	34.2
7	18.7	7960	97,647	98,188	27.4	1.02	746,232	28.4	62.4
8	21.4	14,900	165,038	167,035	18.5	0.6	1,269,466	6.0	13.2
N	8	8	8	8	8	8	8	8	8
Mean	27.2	9863	139,359	144,691	27.1	0.74	1,099,655	18.2	40.1
SD	10.4	2239	36,369	42,159	7.0	0.21	320,408	8.3	18.3
Median									
CV %	38	23	26	29	26	28	29	46	46

(70.3–94.8% of the radioactivity). The major circulating metabolite RO6802287 (M4) was detected in all plasma samples, (5.2–29.7% of the radioactivity), with increasing percentages of M4 versus parent compound over time and thus, M4 would account for more than 10% of the RO5503781-related material in plasma at steady state, and would be classified as a major metabolite according to the MIST guidance. For the patient who dropped out, likewise, parent compound (idasanutlin) was the major drug-related component observed; two metabolites M4 (major) and M2 (minor) were also detected. In individual plasma samples (24 h), parent compound accounted for 84.7%, ranging from 80.4 to 90.2% of the circulating radioactivity, whilst metabolite M4 (RO6802287) accounted for 14.9%, ranging from 9.8 to 19.6%; no significant inter-individual variability in metabolite formation vs parent was observed. The metabolic pathways are shown in Fig. 3.

In feces, unchanged drug idasanutlin was the major drug-related component, accounting for 84.2% of the dose (0–264 h). Since absolute bioavailability is ~40%, which suggests a maximum of 40% of unchanged drug via biliary excretion as the major excretion pathway, another half of 84% unchanged drug in fecal recovery could be the unabsorbed drug. A number of minor drug-related components

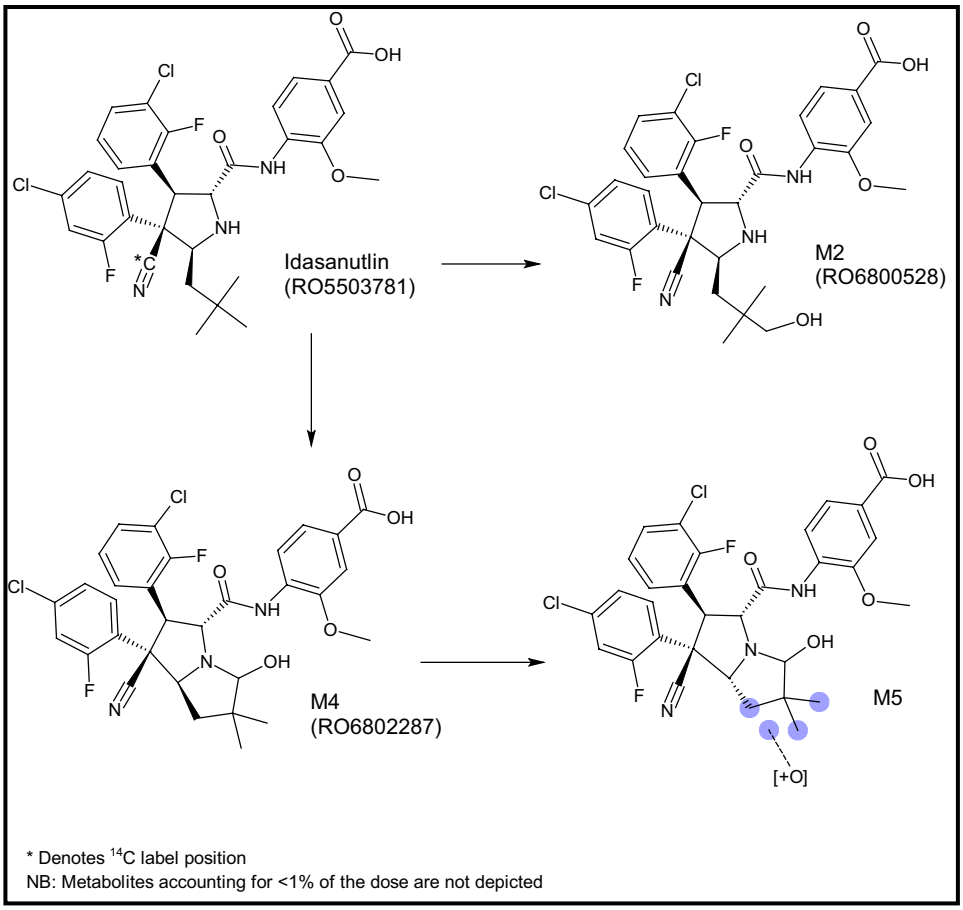

Fig. 3 Proposed major metabolic pathways of ^{14}C-idasanutlin in humans

(Fig. 3) were identified in feces (0–264 h pool), including the hydroxy pyrrolidine metabolite (M4; RO6802287; 3.4% of the dose), a mono-oxidised metabolite (M2; RO6800528; 1.6%) and a product postulated to result from oxidation of metabolite M4 (M5; accounting for 1.6% of the dose). Additional very minor metabolites (accounting for between 0.2 and 0.8% of the dose) were also identified in feces; these mainly resulted from di-oxidation and/or further oxidation of metabolite M2 and/or M4. Overall, hepatic metabolism including glucuronidation was modest at best.

Safety and tolerability

Overall, the idasanutlin treatment was shown to be safe and tolerable. The safety profile for idasanutlin treatment is comparable and consistent with the known safety profile of idasanutlin in patients with solid tumors based on previous idasanutlin clinical experience. No new safety signals were identified.

Palatability assessment (Treatment C)

Due to a premature study withdrawal of the patient who dropped out during Dosing Period 1, only 7 patients (87.5%) completed the palatability assessment. Overall, the patients reported moderately intense taste with some degree of bitterness and unpleasant taste with the idasanutlin SDP formulation. The placebo formulation did not have any intense taste, overall taste, or unpleasant taste.

Efficacy

Efficacy was an exploratory objective in this study. Patients who entered the Optional Treatment Extension and had tumor assessments performed at the second cycle of the Optional Treatment Extension were included in the efficacy analysis population. Only 1 of 8 patients (12.5%) was included in the efficacy analysis population. Tumor response assessment was evaluated according to RECIST v1.1. The best response came from one patient with stable disease reported on Day 56 (Cycle 2) and Day 107 (Cycle 4) of Optional Treatment Extension and progressive disease reported on Day 176 during the final visit.

Discussion

Idasanutlin by oral administration is initially being developed for patients with AML who often have the intrinsic (e.g., renal and/or hepatic impairment) and extrinsic (e.g., drug–drug interaction) factors and thus, these two types of clinical pharmacology studies were deemed relevant and necessary. To have a thorough understanding of the disposition of idasanutlin, which is critical for the evaluation of the effect of intrinsic factors on the exposure of idasanutlin, the current study was conducted.

A mass-balance (human ADME) study using oral [^{14}C]-labeled idasanutlin was conducted to collect blood/plasma, urine, and fecal samples providing the mass balance and metabolite identification data and to determine the need to conduct dedicated trial(s) for organ impairment on PK exposure and/or drug–drug interaction. The estimation of the absolute bioavailability of an oral product further characterized its absorption kinetics and could be achieved through IV administration of [^{13}C] isotopically labeled idasanutlin tracer dose, which allowed the fate of the IV dose to be distinguished from the oral dose by means of an isotopic tracer.

Excretion balance for [^{14}C]-idasanutlin was shown as near complete recovery from 1 to 10 days of collection intervals for all patients. Most of the administered radioactivity was recovered in the first 5 days post-dose (all above 75%), with exception of one patient who needed 11 days post-dose. Total drug-associated radioactivity or unchanged drug was predominantly excreted in the feces (mean of 91.5%), with very little excreted in the urine (mean of < 0.1%). These findings suggest no impact of patient's renal status on PK exposure; on the other hand, they suggest the necessity of investigating the impact of patient's biliary excretion and hepatic function on idasanutlin PK exposure, which can be accomplished either through population PK analysis of phase 1 and phase 3 samples, or via a dedicated hepatic impairment study.

Total radioactivity in plasma and whole blood and PK parameters for idasanutlin and M4 metabolite were assessed for systemic exposure of drug-related materials. Mean C_{max} and $AUC_{0-\infty}$ were 30% lower in whole blood (on average with 45% hematocrit content) compared with plasma, indicating little association of [^{14}C]-radioactivity with red cells.

Plasma balance suggests that the major circulating drug-related compounds were idasanutlin and M4 metabolite, as the two together accounted for all (~ 100%) of circulating total plasma radioactivity, suggesting few other major circulating moieties following administration of idasanutlin. Consistent with the results in the other phase I studies with idasanutlin, the plasma concentration of idasanutlin peaked at < 10 h following oral dose administration, with median t_{max} reached by approximately 8 h. The estimated terminal $t_{1/2}$ values for idasanutlin (~ 1 day) and the M4 metabolite (~ 2 days) were also similar to the results from the other phase I studies [5–7]. The twice longer $t_{1/2}$ value for the M4 metabolite than idasanutlin partially contributes to higher M4 metabolite/idasanutlin ratio (15.8% C_{max} and 29% AUC) than in fecal samples (3.4% amount) that also count unabsorbed drug.

Co-administration of the IV tracer dose (100 µg) with a single oral dose of 100 mg MBP and 300 mg SDP idasanutlin resulted in the absolute bioavailability of idasanutlin SDP of ~ 40%. Physicochemical properties for idasanutlin showed low aqueous solubility and permeability. These data suggest that the SDP formulation provides moderate absolute bioavailability, which is favorable for the oral product.

On the basis of the results in this study, IV kinetics and absolute bioavailability utilizing a tracer-dose isotope approach as well as the metabolic pathway and excretion of idasanutlin, including its major circulating metabolites, are well elucidated and allow for a comprehensive understanding of the disposition of idasanutlin and its M4 metabolite in humans. The add-on study design evaluating both absolute bioavailability and mass balance in each patient enabled full characterization of clinical ADME properties for idasanutlin within the same patient. It is, thus, reliably identified that the inter-patient variability of the PK exposure parameters following IV administration was approximately half following oral administration, suggesting major contribution of oral absorption in variability.

A moderate V_d of 27 L observed following IV administration of [^{13}C]-idasanutlin is lower than total body water (42 L) for a 70-kg adult. However, due to bi-exponential nature of the disposition curve, the initial quick decline of the blood level suggests extensive distribution of idasanutlin into tissues, which is consistent with the lipophilic nature of the molecule and preclinical tissue distribution studies showing high levels of idasanutlin-associated radioactivity in all tissues [2]. This high distribution into relevant tissues could be favorable given the high unmet need for the treatment of AML in bone marrow.

The IV administration of [^{13}C]-idasanutlin also demonstrated a low mean plasma CL_t of 0.74 L/h. The observed C_{max} is tenfold higher than predicted (based on non-compartmental analysis), further suggesting that the hepatic extraction ratio of idasanutlin is low. As previously reported, no effect of food was apparent on the pharmacokinetics of idasanutlin and co-administration of a potent CYP3A inhibitor had no change on C_{max} and only had a modest effect (~ 31% increase) on idasanutlin AUC [7], supporting minimal first-pass metabolism of idasanutlin.

The unusually slow elimination of the M4 metabolite contributed to higher exposure of blood and plasma

radioactivity (much less of unchanged idasanutlin) in the patient who dropped out (Subject 3). It is interesting to note that this patient had bile duct cancer. Bile duct is an important organ for drug excretion and elimination, e.g., glucuronidation, a pathway for idasanutlin/M4 metabolism. Therefore, this patient's disease status might have contributed to the unusual PK finding.

The palatability assessment was conducted during Dosing Period 2 to assess the palatability of the future pediatric oral formulation of idasanutlin. Overall, the placebo formulation did not have any intense taste, overall taste, or unpleasant taste. The idasanutlin SDP formulation was reported to have some moderate degree of bitterness and unpleasant taste. Appropriate flavoring agents and sweeteners may be considered to be added for taste masking in the future pediatric oral formulation.

Together, the data generated in this study enabled a thorough understanding of the disposition of idasanutlin. Co-administration of an oral dose of idasanutlin with an IV tracer dose revealed a low systemic CL and a moderate absolute bioavailability of idasanutlin. Idasanutlin and its major inactive metabolite, M4, were the major circulating moieties in plasma, and excretion of idasanutlin-associated radioactivity was primarily via the fecal route, with negligible amounts recovered in urine, following oral administration. The clinical implications of this study support the conclusion that renal impairment is unlikely to significantly impact exposure to idasanutlin and M4 metabolite, whereas significant hepatic impairment may potentially alter exposure to the parent drug and/or metabolite(s). The potential for drug–drug interactions is low.

Acknowledgements The authors would like to acknowledge key contributions from Roche colleagues, investigational site staff, and patient volunteers.

Funding The study was sponsored by F. Hoffmann-La Roche. The funding sources had no role in the design, analysis, and interpretation of the results, and thus the authors were independent from the funding source.

Compliance with ethical standards

Conflict of interest LC, SB, FV, RJ, and JZ are employees of Roche. DD was an employee of Rutgers University, through a fellowship grant from Roche.

References

1. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, Kong N, Kammlott U, Lukacs C, Klein C, Fotouhi N, Liu EA (2004) In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 303:844–848. https://doi.org/10.1126/science.1092472
2. Ding Q, Zhang Z, Liu JJ, Jiang N, Zhang J, Ross TM, Chu XJ, Bartkovitz D, Podlaski F, Janson C, Tovar C, Filipovic ZM, Higgins B, Glenn K, Packman K, Vassilev LT, Graves B (2013) Discovery of RG7388, a potent and selective p53–MDM2 inhibitor in clinical development. J Med Chem 56(14):5979–5983
3. Higgins B, Glenn K, Walz A, Tovar C, Filipovic Z, Hussain S, Lee E, Kolinsky K, Tannu S, Adames V, Garrido R, Linn M, Meille C, Heimbrook D, Vassilev L, Packman K (2014) Preclinical optimization of MDM2 antagonist scheduling for cancer treatment by using a model-based approach. Clin Cancer Res 20:3742–3752
4. Glenn KJ, Yu LJ, Reddy MB, Fretland AJ, Parrott N, Hussain S, Palacios M, Vazvaei F, Zhi J, Tuerck D (2015) Investigating the effect of autoinduction in cynomolgus monkeys of a novel anticancer MDM2 antagonist, idasanutlin, and relevance to humans. Xenobiotica 19:1–10
5. Siu L, Italiano A, Miller W, Blay J, Gietema J, Bang Y, Mileshkin L, Hirte H, Reckner M, Higgins B, Jukofsky L, Blotner S, Zhi J, Middleton S, Nichols G, Chen L. Phase 1 dose escalation, food effect, and biomarker study of RG7388, a more potent second-generation MDM2 antagonist, in patients (pts) with solid tumors. J Clin Oncol; 2014. In: ASCO annual meeting; May 30–June 3, 2014; Chicago, IL, USA. 2014. p. 5s (suppl; abstr 2535)
6. Yee K, Martinelli G, Vey N, Dickinson MJ, Seiter K, Assouline S, Drummond M, Yoon S, Kasner M, Lee J, Kelly KR, Blotner S, Higgins B, Middleton S, Nichols G, Chen G, Zhong H, Pierceall WE, Zhi J, Chen L (2014) Phase 1/1b study of RG7388, a potent MDM2 antagonist, in acute myelogenous leukemia (AML) patients (Pts). Blood 124(21):116
7. Nemunaitis J, Young A, Ejadi S, Miller W, Chen L-C, Nichols G, Blotner S, Vazvaei F, Zhi J, Razak A (2018) Effects of posaconazole (a strong CYP3A4 inhibitor), two new tablet formulations, and food on the pharmacokinetics of idasanutlin, an MDM2 antagonist, in patients with advanced solid tumors. Cancer Chemother Pharmacol 81(3):529–537
8. Recommendations of the International Commission on Radiological Protection. ICRP Publication 105. Ann ICRP 37(5)