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ORIGINAL ARTICLE

Clinical pharmacology characterization of RG7112, an MDM2 antagonist, in patients with advanced solid tumors

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Abstract
Purpose RG7112, the first selective small-molecule MDM2 antagonist in clinical testing, is a non-genotoxic oral p53 activator. To optimize its dose and schedule, a number of clinical pharmacology characteristics were explored in this multicenter trial in patients with advanced solid tumors.
Method In part 1, the impact of high-energy/high-fat meal and formulations (crystalline and amorphous) on relative bioavailability was examined in single-dose crossover designs. In part 2, schedule optimization (4 schedules of drug administration under fasting condition and 2 cohorts with liquid supplementation) was investigated in parallel, dose escalation designs. Clinical endpoints were pharmacokinetics (PK), pharmacodynamics (PD) including MIC-1 elevation and platelet reduction, and safety/tolerability.
Results With a single-dose treatment, a high-fat/high-energy meal and a new formulation under fasting condition, respectively, enhanced overall bioavailability of RG7112 slightly over twofold. Following multiple-dose administrations, all four schedules yielded the comparable per-cycle (28-d) exposure (AUC), as designed; liquid supplements also enhanced bioavailability. High-dose treatments of consecutive daily dosing for 5 and 3 days resulted in higher on-treatment-day exposure to RG7112 than both weekly and low-dose/long-duration (20-day) daily schedules. Serum MIC-1 and blood platelet profiles showed similar patterns to those of PK when the clinical pharmacology conditions were varied, suggesting the relative importance of treatment-day exposure than overall per-cycle AUC.
Conclusion Food (both high-fat and low-fat meals) and new formulation enhanced bioavailability. High-dose consecutive daily treatment for 3–5 days is superior to weekly and low-dose/long-duration (20-day) daily schedules in yielding the sufficiently high drug exposure and PD effects potentially required for cancer treatment efficacy.

Keywords MDM2 antagonist · Food effect · Formulation change · Schedule optimization

Partial results presented at AACR 2013.

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Introduction

The tumor suppressor p53, which induces cell cycle arrest and apoptosis under stresses, plays a pivotal role in protection from cancer development. In non-stressed cells, the level of p53 is controlled tightly by MDM2 through a negative feedback loop; 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 growth arrest and/or deficient apoptosis. Therefore, blocking the p53–MDM2 interaction is expected to overcome the oncogenic consequences of MDM2 overproduction and restore the p53 function [1].

Small-molecule MDM2 antagonists block p53-MDM2 binding, stabilize p53, and activate p53 signaling. For maximal activities, MDM2 antagonism requires wild-type p53 and restores p53 function in cancers overproducing MDM2 (through amplification and/or overexpression). The tumor and soluble biomarkers include: elevation of p53 and p21 expression, Ki-67 for decreased cell proliferation, TUNEL for apoptosis, MDM2 mRNA, and serum macrophage inhibitory cytokine-1 (MIC-1). Of those, MIC-1 is the most convenient one to be sampled and expected to show increase with MDM2 antagonist treatment.

RG7112 is a representative of the Nutlin family of small-molecule MDM2 antagonists. The compound has good oral bioavailability, and it has shown tumor growth inhibition and regression in 4/4 mouse xenograft models of human cancer at doses that did not cause significant toxicity [2–4]. RG7112 was the first of the Nutlin class of MDM2 antagonists tested in humans [5], and it has demonstrated p53 pathway mechanism of action (MOA) in patients with MDM2-amplified liposarcoma [6]. Biomarker responses were consistent with the drug acting on the desired molecular target; the increased pharmacodynamics (PD) responses were significantly correlated with exposure (AUC) to RG7112 following drug administration of the maximum tolerated dose (MTD) QD daily × 10 days. The threshold level of MIC-1 elevation suggests that a minimum exposure of 50,000 ng h/mL of AUC [5] is required to yield a meaningful MIC-1 change. Currently, however, we do not yet know any association between MIC-1 elevation and tumor shrinkage or clinical response/efficacy.

In evaluating the product profile, several potential liabilities are emerging for RG7112 molecule: A high dose is required with pill burden that impacts GI intolerance, a delayed thrombocytopenia leads to underexposure required for efficacy, etc. The two potential mechanisms have been identified to result in thrombocytopenia through RG7112-induced disruption of MDM2–p53 interaction: (1) increase in apoptosis during the early stages of megakaryocytes (MK) maturation and (2) impairment of the endomitotic process and polyploidization during the later stages of MK maturation [7]. The use of RG7112 for the treatment of solid tumors might have to utilize a schedule which minimizes this potential toxicity.

This comprehensive clinical pharmacology study aimed to improve dosing condition and optimize dose/exposure and treatment schedules. This was a 2-part phase I study to examine the potential effect of food and formulations on the relative bioavailability of the MDM2 antagonist RG7112 (part 1) and to examine the effect of RG7112 doses and schedules on pharmacokinetics (PK), pharmacodynamics (PD), as well as safety and tolerability (part 2), in patients with solid tumors.

Materials and methods

Overall study design

This was an open-label, two-part study performed at five centers in the USA. RG7112 was supplied as oral film-coated tablets as follows: phase 1 crystalline formulation (micronized free base), amorphous formulation (prototype), new crystalline formulation (milled free base), and new amorphous formulation (final). Overall study design for both parts is summarized in Fig. 1.

Part 1 consisted of three treatment stages that occurred in sequential order with six different patients planned for each stage. Each patient participated in an initial relative bioavailability assessment cycle (Cycle 1) with a randomized sequence of three single-dose treatments of RG7112; patients who did not complete their assigned three single-dose treatments for any reason were replaced. In stages 1 and 2, three 1500 mg RG7112 treatments given, separated by a washout period of at least 7 days, were designated as crystalline formulation under fasted (reference standard) and fed conditions, with the third treatment of either amorphous formulation (prototype) in stage 1 or new crystalline formulation in stage 2 under fasted conditions. With this design, the sample size for crystalline formulation with or without food was doubled to $N = 12$. In stage 3, another three RG7112 treatments given, separated by a washout period of at least 9 days, were designated as 1500 and 2500 mg doses under fasted conditions and 2500 mg dose under fed condition of new amorphous formulation (final).

Following Cycle 1, patients in stages 1 and 2 had the option to continue therapy (1500 mg of phase 1 crystalline formulation daily × 10d with or without food based on the patient's preference, followed by 18 days of rest per cycle) in additional 28-day treatment cycles (extension phase) until either clinically defined progressive disease and/or intolerable toxicities. If stage 3 patients chose to be treated in the extension phase, from Cycle 2 onward, they were treated with 28-d cycles of 1500 mg new amorphous formulation daily × 3d under fasting condition, followed by 25 days of rest per cycle, until either disease progression and/or intolerable toxicity. During treatment Cycle 2, PK samples were also collected following doses on days 1 through 3 to lead in the daily × 3d schedule for part 2.

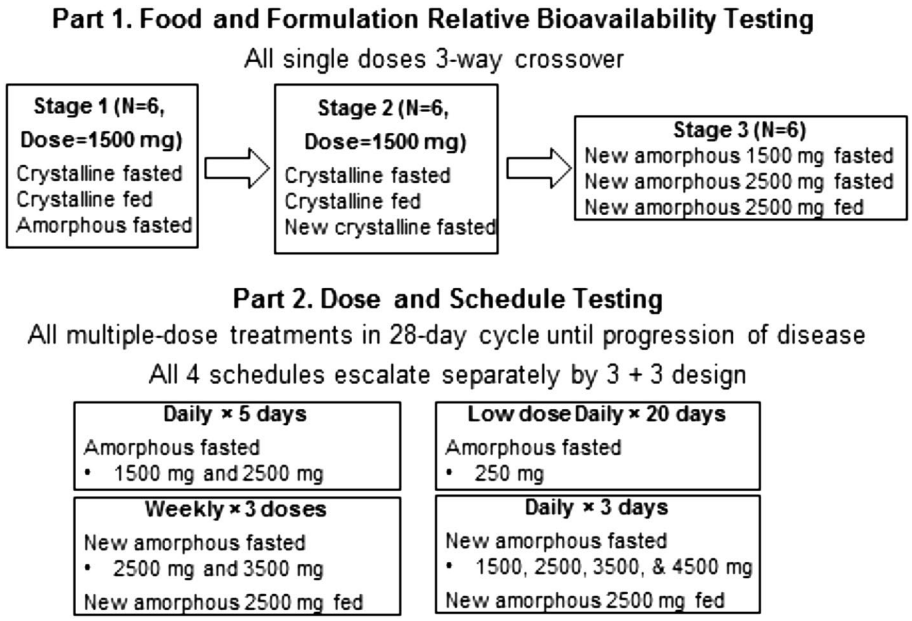

Fig. 1 Overall study design

In part 2, patients received escalating multiple doses of RG7112 in four separate treatment schedules consisting of cycles of 28 days. Patients in daily × 5d (starting dose 1500 mg) and daily × 20d (250 mg) schedules received amorphous formulation (prototype), under fasted condition. Patients in weekly × 3 doses (starting dose 2500 mg) and daily × 3d (starting 2500 mg) schedules received new amorphous formulation (final). For the latter two schedules, the first 3 patients enrolled were dosed under fasted conditions, and subsequent cohorts could have included both fasted and fed conditions to assess food (in liquid supplementation) effect on PK and PD.

For each part 2 schedule, at the end of the first 28-day treatment cycle, patient safety, tolerability, and PK data were reviewed for determination whether further dose escalation cohort up to a maximum tolerated dose (MTD) was warranted or until dose-limiting toxicity (DLT) criteria were rendered, should the treatment-day exposure target [5] not be reached.

Ethics and study conduct

The study was conducted in accordance with the principles of Good Clinical Practice. Institutional Review Boards (IRBs) and/or Independent Ethics Committee (IECs) approvals were obtained from all 5 participating investigational sites.

Selection of study population

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: NCT01164033).

Concomitant medications and dietary restriction

RG7112 is metabolized mainly by and is a moderate competitive inhibitor of CYP3A4/5; it is also an inhibitor and weak substrate of P-glycoprotein (P-gp). Therefore, the concomitant administration of compounds that are known substrates of P-gp and substrates, inhibitors, or inducers of CYP3A4/5 was to be avoided unless deemed essential by the investigator for patient safety or due to diminished efficacy. Concomitant use of medications known to have the potential to cause QT prolongation, under "Risk of TdP" and "Congenital QT" [8], was prohibited. Medications listed as "Possible risk of TdP" and "Congenital TdP" [8] were allowed only when medically necessary and no suitable alternatives were available. Herbal remedies such as St. John's Wort, Ginkgo biloba, traditional Chinese medicines, and foods containing caffeine and grapefruit juice were also avoided.

Assessments

Blood samples for plasma concentrations of RG7112 and serum macrophage inhibitory cytokine-1 (MIC-1) levels were collected at the same time points at baseline (control) and periodic post-dose time points following drug administration on assessments days. Plasma PK samples were analyzed for RG7112 [6] by LC/MS method (Tandem Labs, West Trenton, New Jersey), while serum PD samples

were analyzed by enzyme-linked immunosorbent assay for MIC-1 (Caris Science Inc., Irving, TX).

The efficacy was assessed for tumor response according to the Response Evaluation Criteria in Solid Tumors (RECIST) 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

As this was a phase I study, no formal statistical hypothesis testing was planned other than thorough PK/PD relation analyses. The determination of the sample size was thus based on practicality.

The key PK parameters such as AUC_{τ} (where τ is dosing interval), AUC_{last}, $AUC_{0\text{-}\infty}$, C_{max}, t_{max}, and $t_{1/2}$ were derived from RG7112 plasma concentration–time profiles using Phoenix WinNonlin v6.2/PKS v4.0.2 software (Certara, Princeton, NJ) that included non-compartmental analysis, plotting and tabulating, and descriptive statistics wherever appropriate. The per-cycle AUC was estimated by multiplying AUC_{τ} with the number of doses per 28-day treatment cycle.

Serum MIC-1 levels were converted to change from baseline (CfBL) prior to calculations of peak concentrations and AUC similar to PK. Platelet levels were also analyzed by normalizing to patient baseline counts.

Results

Study population

Demographic data were summarized in Table 1. A total of 76 patients (42 F and 34 M, mean age 60 years) with solid tumors including lymphoma were enrolled and treated in either part 1 ($N = 23$) or part 2 ($N = 53$). In the 1st cycle of treatment, 36 patients received weekly × 3 (26 with food/formulation evaluation), 15 patients daily × 5d, 6 patients daily × 20d, and 19 patients daily × 3d (three

Table 1 Summary of demographic data by treatment

Demos	Part 1 (single dose)		Part 2 (multiple dose)			
	S1&2 ($N = 15$)	S3 ($N = 7$)	2A ($N = 15$) daily × 5d	2B ($N = 6$) daily × 20d	2C ($N = 13$) weekly × 3	2D ($N = 19$) daily × 3d
Sex						
Male	9 (60 %)	4 (57 %)	8 (53 %)	2 (33 %)	4 (31 %)	7 (37 %)
Female	6 (40 %)	3 (43 %)	7 (47 %)	4 (67 %)	9 (69 %)	12 (63 %)
Race						
Asian	–	–	–	–	–	1 (5 %)
Black	1 (7 %)	1	4 (27 %)	–	–	4 (21 %)
White	14 (93 %)	6	11 (73 %)	6	12 (92 %)	14 (74 %)
Other	–	–	–	–	1 (8 %)	–
Age in years						
Mean	56.7	64.9	60.5	61.8	58.5	62.6
SD	15.0	14.1	9.3	7.2	10.6	13.6
Median	60	66	61	61	59	64
Min–max	24–80	43–88	34–70	54–70	41–74	29–81
Weight in kg						
Mean	75.95	69.80	84.56	92.50	76.19	71.75
SD	19.81	17.59	25.88	8.55	23.68	18.51
Median	72.8	69.9	83.6	92.9	67.0	71.1
Min–max	47.9–117.8	49.4–99.1	55.8–159.3	82.8–104.7	53.5–139.6	41.3–117.8
Height in cm						
Mean	169.7	173.3	173.4	163.3	162.8	167.1
SD	9.83	10.11	9.88	11.40	9.18	10.34
Median	170	178	173	164	162	167
Min–max	152–184	161–187	159–191	145–175	152–178	145–185

N represents number of patients contributing to summary statistics. Percentages are based on *N* (number of valid values). *S* stage

with food evaluation) doses. Except for the low-dose 250 mg/day, daily doses ranged narrowly from 1500 to 4500 mg of RG7112. The majority of patients were Caucasian women.

Of the 76 patients, 51 were initially diagnosed with local regional disease and 25 were diagnosed with metastatic diseases. There were no patients with non-Hodgkin's lymphoma. All patients received prior concomitant treatment for their cancer and had other concurrent disease at the time of enrollment.

The best clinical response was seen from part 2, where stable disease was achieved in 6 patients, evenly distributed to all four schedules (2 each for daily × 5d and 3d; 1 each for daily × 20d and weekly × 3).

Pharmacokinetics and pharmacodynamics

Food and formulation effects on relative bioavailability of RG7112

Similar patterns were observed between plasma RG7112 (upper Fig. 2) and serum MIC-1 (lower Fig. 2) concentration–time profiles following single doses: A high-energy/high-fat meal enhanced PK exposure and MIC-1 response for the current crystalline formulation, and amorphous formulation performed better than crystalline formulation when both were administered under fasting condition. The food effect on increased exposure was also demonstrated for amorphous formulation with single-dose (Supplement

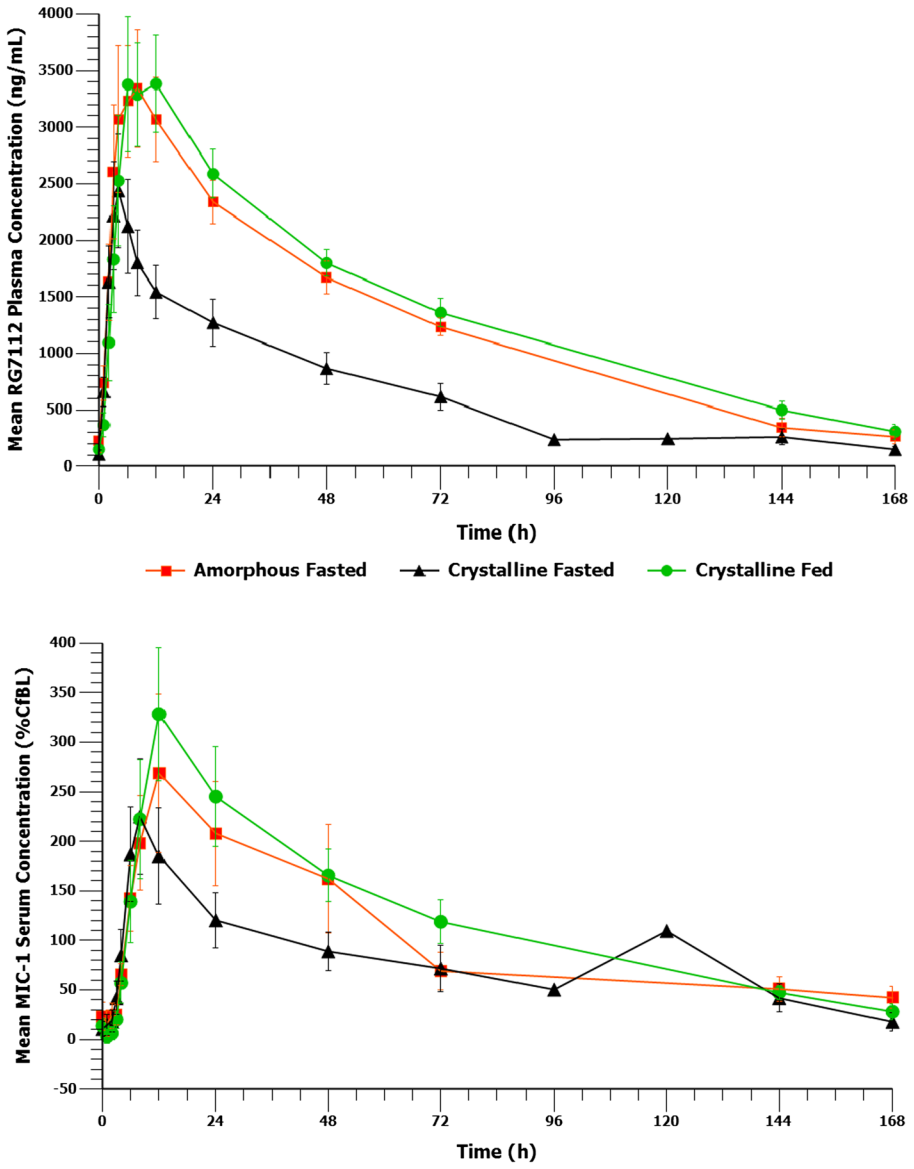

Fig. 2 Mean (±SE) plasma RG7112 (*upper*) and serum MIC-1 (*lower*) concentration–time profiles following single dose of RG7112 administrations with crystalline formulation fasted and fed as well as amorphous formulation fasted. *CfBL* change from baseline, *MIC-1* macrophage inhibitory cytokine-1

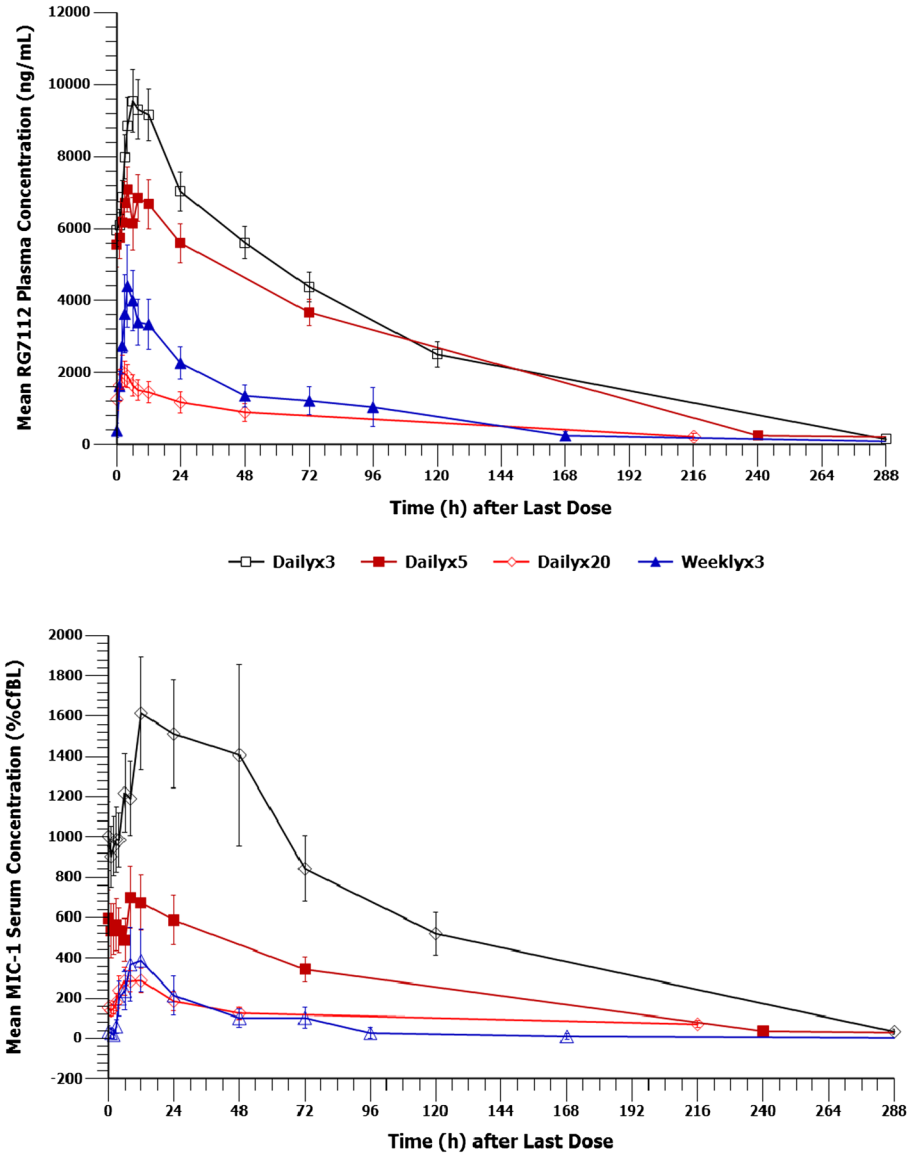

Fig. 3 Mean (±SE) plasma RG7112 (*upper*) and serum MIC-1 (*bottom*) concentration–time profiles on the last day of treatment from the 4 tested multiple-dose schedules (daily × 5d, daily × 20d, weekly × 3, and daily × 3d). *CfBL* change from baseline, *MIC-1* macrophage inhibitory cytokine-1. *Note*: All doses were combined for each schedule, including liquid supplement cohorts. For daily × 3d schedule, one patient with extremely high MIC-1 was excluded

1) and multi-dose drug administration with a liquid food supplement (approximately 500 kcal with approximately 30 g of fat) for the weekly × 3 and daily × 3d schedules (Supplement 2).

Schedule dependency on PK exposure, PD MIC-1, and safety platelet profiles

For part 2, Fig. 3 summarizes last-day plasma RG7112 (upper) and serum MIC-1 (lower) concentration–time profiles following repeated dosing schedules of 1st treatment cycle; PK parameters are summarized in Supplement 2. While high-dose daily schedules (×5d and ×3d) yielded high peak RG7112 concentrations and MIC-1 levels on the last day of dosing due to drug and MIC-1 accumulations, these were less accumulated for the weekly and low-dose daily × 20d schedules even though their per-cycle AUC exposures were comparable to or higher (Supplement 2). A relational analysis between AUCs of RG7112 and MIC-1 (Fig. 4) further confirms a potential scheduling dependency between daily and weekly schedules. These PK/PD differences between the schedules have been translated to varying platelet concentration–time profiles (Fig. 5): high-dose daily × 5d and × 3d schedules were the most potent, causing pronounced platelet count drops (>30 %) from baseline with similar nadir timing.

Safety

The vast majority of patients (>97 %) reported at least 1 AE in either part 1 or part 2 of the study. The most commonly reported AEs were drug-related GI distress including

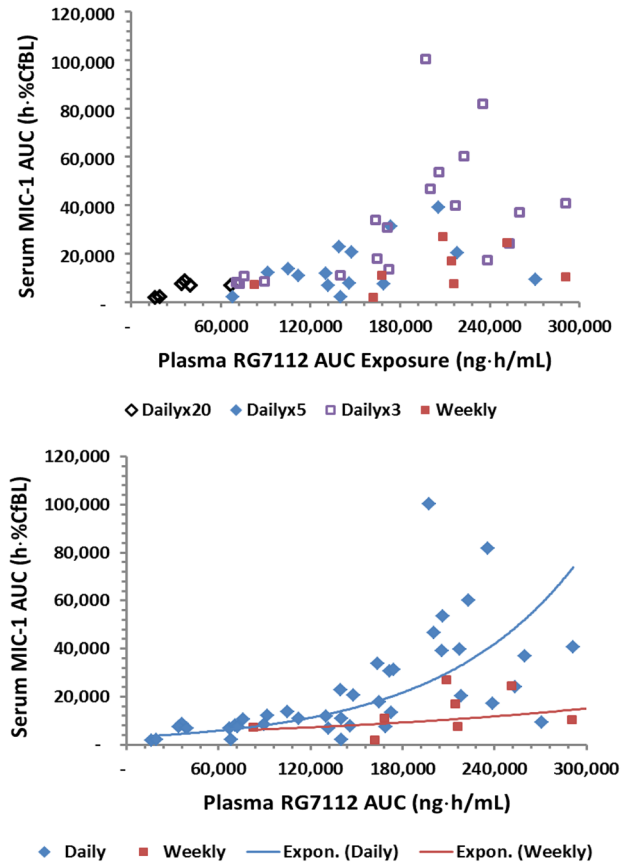

Fig. 4 Exposure–effect relationship between plasma RG7112 and serum MIC-1 AUCs (*upper*) on the last day of treatment from the 4 tested multiple-dose schedules (daily × 5d, daily × 20d, weekly × 3, and daily × 3d), fitted with an empiric exponential model for daily combined and weekly schedules, respectively (*lower*). *CfBL* change from baseline, *MIC-1* macrophage inhibitory cytokine-1. AUC values are AUC24 h for all daily schedules and AUCinf for the weekly schedule. For clarity, two data points with extremely high MIC-1 and exposure are not shown

anorexia, nausea, vomiting, diarrhea, and/or abdominal pain. The majority were grades 1 and 2.

After single-dose treatment in part 1, Cycle 1, the tolerability was similar for the formulations and food effect tested. Neither the SAEs (2 patients receiving amorphous formulation with cardiac tamponade, gastric ulcer, plural effusion) nor the AE leading to withdrawal (1 patient receiving amorphous formulation) was considered related to study treatment. During the optional treatment extension phase of part 1, tolerability was also similar for the formulations tested (crystalline and amorphous). AEs were reported by 82 % of patients in crystalline formulation ($N = 11$) and 100 % ($N = 5$) of patients in new amorphous formulation. The type and incidence of AEs were similar in both treatment groups, with the exception of nausea and vomiting, which had a higher incidence with the crystalline formulation (4 of 11 patients reported each AE) than with the new amorphous formulation (1 of 5 patients with nausea but none with vomiting). While no SAEs were reported during the optional treatment extension phase, 2 (18 %) patients reported severe AEs (both with thrombocytopenia, crystalline formulation). Three patients (all crystalline) were withdrawn due to AEs (2 with thrombocytopenia and 1 with nausea), all of which were considered related to study treatment.

In part 2, 52 patients (98 %) experienced at least 1 AE. While most patients in part 2 had AEs of mild (≥92 %) or moderate intensity (≥85 %), approximately one-third of patients in all treatment schedules also had a severe AE, with the highest proportion in the most intense (high-dose daily) treatment regimen (42 % of patients in daily × 3d schedule). Life-threatening AEs were experienced by 3 patients (20 %) in daily × 5d schedule, 1 patient (17 %) in low-dose daily × 20d schedule, and 1 patient (8 %) in weekly schedule. Twenty-one SAEs were reported by 13 patients, 5 of whom had SAEs considered potentially related to study treatment (1 patient with G4 thrombocytopenia, anemia, and neutropenia; 1 patient with G3 delirium), with 3 of these 5 patients in daily × 5d schedule. Six patients were withdrawn due to AEs: 3 in daily × 3d schedule and 1 in each of all other 3 schedules.

Hematology

In part 1, mean hemoglobin counts remained ≥9.0 g/dL and mean platelet counts remained ≥100.0 × 10^9/L in all treatment groups at all time points. Two patients in stages 1 and 2 combined had platelet levels to be considered being severe (CTCAE Grade 3). The only other hematology parameter to have Grade 3 or 4 values during part 1, Cycle 1 was low lymphocyte levels: 2 patients in stages 1 and 2 combined and 1 patient in stage 3 had lymphocyte levels of Grade ≥3.

For the majority of patients in part 2, the worst severity for the hematological laboratory parameters was Grade 0 or 1. However, some values of a Grade ≥3 were reported, and, overall, the two high-dose daily schedules had the highest incidence of Grade ≥3 hematological values (Supplement 3). The most frequently reported Grade ≥3 hematology parameter was low lymphocytes: 7 patients (47 %) in daily × 5d schedule and 6 patients (32 %) in daily × 3d schedule. Low platelet levels of Grade 4 severity were reported for 2 patients with in daily × 5d schedule; no other Grade ≥3 reports of low platelet levels were reported in the other 3 treatment schedules.

Discussion

This study consisted of two parts: Part 1 comprised an initial single-dose, crossover assessment followed by a multiple-dose optional treatment extension with a constant dose,

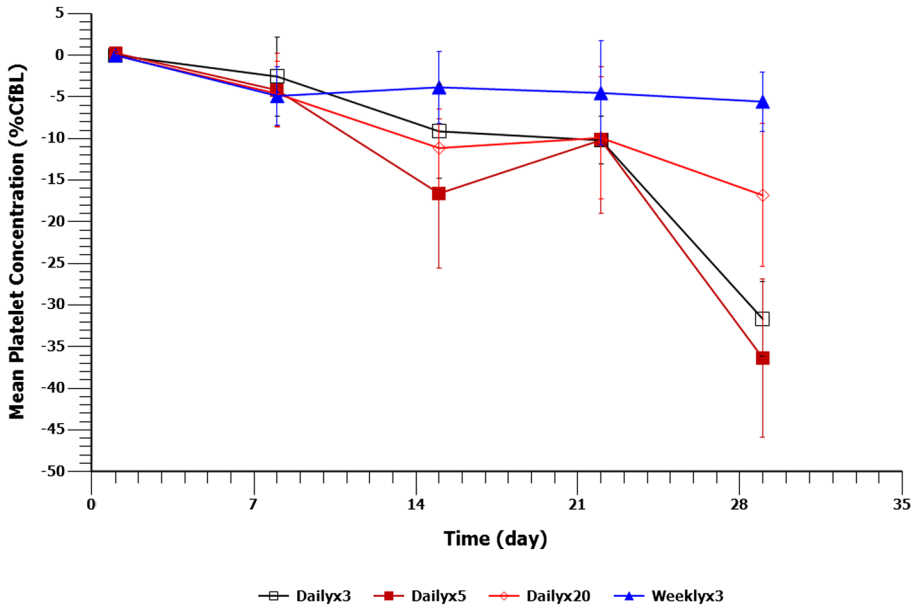

Fig. 5 Mean (±SE) platelet concentration–time profiles from the 4 tested multiple-dose schedules (daily × 5d, daily × 20d, weekly × 3, and daily × 3d). *CfBL* change from baseline

while part 2 comprised four different treatment schedules of escalating doses of RG7112.

In part 1, the primary objectives were to examine the potential food effect on the relative bioavailability of RG7112 with its crystalline and amorphous formulations. An efficient design for stages 1 and 2 was deliberate: using only 12 evaluable patients, one definitive food effect of the current formulation and two exploratory evaluations for the two new formulations were accomplished. The results from the current study suggest that a high-fat (approximately 50 g) and high-energy (approximately 1000 kcal) meal enhanced overall bioavailability slightly over twofold for both tested formulations. The food effect was further explored with two multiple-dose regimens (QD × 3d and weekly × 3) in part 2 with a liquid food supplement mimicking "regular diet" (approximately 30 g of fat and 500 kcal energy). Thus, both high-energy/high-fat and "regular diet" food doubled exposure and halved variability for both crystalline and amorphous formulations. Higher systemic exposure is not unexpected for poorly soluble compounds such as RG7112 when given in the fed state. Food induces various physiological changes in the gastrointestinal (GI) environment, which yield higher levels of native surfactant, the presence of lipophilic meal components, the presence of the products of fat digestion, larger volumes of fluids available to dissolve the drug, and longer upper-GI residence time, yielding more rapid and extensive dissolution under fed conditions. A similarly enhanced exposure to RG7112 was seen with an amorphous formulation administered under fasted condition, suggesting an intrinsic improvement in relative bioavailability with amorphous formulation. RG7112 molecule is a weak base, and its solubility is pH dependent. Variations in upper-GI pH influence in vivo dissolution behavior of weak bases; changing polymer in the amorphous formulation reduces pH dependency and yields high bioavailability even under fasted conditions.

Based on phase I data for RG7112 [5], thrombocytopenia was dose limiting on a continuous 10-day schedule for patients in the highest one-third of exposures. The 10-day schedule also led to platelet nadirs at or after day 28, significantly delaying dosing of subsequent cycles. Modeling of these data suggested that thrombocytopenia could be ameliorated by a shorter schedule (although it could still be significant as doses are escalated), while maintaining efficacy, as non-clinical models suggest that achieving a high RG7112 maximum plasma concentration (C_{max}) may be critical for efficacy. Timing of onset and recovery of thrombocytopenia could also be altered by a shorter schedule, creating the possibility of more consistent timing of subsequent cycles. In part 2, safety was thus the primary objective and we examined the effect of the dose and dose schedule of RG7112 on patient safety and tolerability including the effect on individual patient platelet counts; secondary objectives were to assess the PK and PD of RG7112, as well as clinical responses, if any. Even though the sample sizes may be limited, we hoped to address the following questions: (1) Can a shorter schedule be escalated more safely to reach higher daily exposures than a 10-day schedule? (2) Can a longer-/lower-dose schedule separate efficacy/toxicity? (3) How does treatment daily × 3d compare with weekly × 3 schedule?

Our strategy was to safely dose-escalate to achieve similar exposure (AUC) per cycle for all of the potential schedules explored. Part 2 pharmacokinetic results confirmed that, as designed, all four tested schedules yielded similar AUC per cycle with varying treatment-day exposure

including C_{max} and AUC: significantly higher with high-dose daily × 3d and × 5d schedules than weekly and low-dose daily × 20d schedules. A trade-off for a shorter treatment schedule might be the potential negative impact on efficacy. Using serum MIC-1 as a pharmacodynamic marker of p53 pathway activation and as a surrogate for induction of apoptosis, the potential for schedule dependency was examined. Comparison of the four tested schedules reveals some interesting distinctions or similarities with each other. It shows that consecutive daily dosing yielded highest MIC-1 elevation regardless of day 3 or day 5; in contrast, neither weekly nor low-dose/long-duration schedules yielded sufficiently high MIC-1 elevation. Limited efficacy data in stable diseases (>6 cycles of therapy) suggest a slight advantage of clinical benefit for daily × 3d and × 5d high-dose schedules with RG7112 treatment.

Using the safety marker of cumulative effect of p53 activation, i.e., platelet reduction, preclinical toxicology data suggested dose-dependent, delayed cytopenia including thrombocytopenia. The clinical data from the present study (part 2) with the tested 4 alternative schedules on the depth and timing of platelet nadir indicated that the high-dose daily (× 5d and × 3d) schedules are the most potent ones for causing pronounced platelet drop (>30 %) from baseline; the nadir timing was similar. Hence, scheduling changes may not be sufficient to overcome hematological impairment such as thrombocytopenia. Of the 4 dosing regimens tested, weekly schedule, even though the least pharmacologically active (in MIC-1 elevation), was the only treatment schedule that did not have any reports of thrombocytopenia as an AE, and there were no reports of low platelet levels above Grade 1.

In conclusion, RG7112 was generally well tolerated with GI toxicities (nausea and vomiting) being the most common AEs, and these were treatable with anti-emetics. Lower dose for longer periods and weekly schedules did alleviate the hematopoietic effects of RG7112, but neither exhibited adequate p53 activation, as suggested by lower levels of the MIC-1 biomarker in patients. On the other hand, high-dose consecutive daily dosing appears to be sufficient to maintain the desired treatment-day exposure including C_{max} and AUC at steady state and PD effects potentially required for cancer treatment efficacy. However, its increased hematological toxicities need to be managed for a successful cancer therapy, such as through combination therapies with concomitant medications that do not have significant effects on hematological reductions.

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Compliance with ethical standards

Conflict of interest GN, SM, AB, NS, RP, and JZ are employees of Hoffmann-La Roche. All other authors report no potential conflict of interest for the work under consideration.

References

1. Chene P (2003) Inhibiting the p53-MDM2 interaction: an important target for cancer therapy. Nat Rev Cancer 3:102–109
2. Vu B, Wovkulich P, Pizzolato G, Lovey A, Ding Q, Jiang N, Liu J-J, Zhao C, Glenn K, Wen Y, Tovar C, Packman K, Vassilev L, Graves B (2013) Discovery of RG7112: a small-molecule MDM2 inhibitor in clinical development. ACS Med Chem Lett 4:466–469
3. Tovar C, Graves B, Packman K, Filipovic Z, Higgins B, Xia M et al (2013) MDM2 small-molecule antagonist RG7112 activates p53 signaling and regresses human tumors in preclinical cancer models. Cancer Res 73:2587–2597
4. 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
5. Kurzrock R, Blay J-Y, Bui BN, Wagner AJ, Maki RG, Schwartz GK, Patnaik A, Gore L, Wu L, Vassilev LT, Ding M, Geho D, Zhi J, Middleton S, Nichols GL (2012) A phase I study of MDM2 antagonist RG7112 in patients with relapsed/refractory solid tumors. ASCO 2012 Abstract
6. Ray-Coquard IL, Blay J-Y, Italiano A, Le Cesne A, Penel N, Zhi J, Heil F, Rueger R, Graves B, Ding M, Geho D, Middleton SA, Vassilev L, Nichols GL, Bui BN (2012) Effect of the MDM2 antagonist RG7112 on the p53 pathway in patients with MDM2-amplified well-differentiated or dedifferentiated liposarcoma: an exploratory proof-of mechanism study. Lancet Oncol 13(11):1133–1140
7. Iancu-Rubin C, Mosoyan G, Glenn K, Gordon RE, Nichols GL, Hoffman R (2014) Activation of p53 by the MDM2 inhibitor RG7112 impairs thrombopoiesis. Exp Hematol 42(2):137–145
8. Revised 24 Jul 2009, Source www torsades org