

Results of the Phase I Trial of RG7112, a Small-Molecule MDM2 Antagonist in Leukemia

Michael Andreeff¹, Kevin R. Kelly², Karen Yee³, Sarit Assouline⁴, Roger Strair⁵, Leslie Popplewell⁶, David Bowen⁷, Giovanni Martinelli⁸, Mark W. Drummond⁹, Paresh Vyas¹⁰, Mark Kirschbaum⁶, Swaminathan Padmanabhan Iyer², Vivian Ruvolo¹, Graciela M. Nogueras González¹, Xuelin Huang¹, Gong Chen¹¹, Bradford Graves¹², Steven Blotner¹¹, Peter Bridge¹¹, Lori Jukofsky¹¹, Steve Middleton¹¹, Monica Reckner¹¹, Ruediger Rueger¹³, Jianguo Zhi¹¹, Gwen Nichols¹¹, and Kensuke Kojima¹

Abstract

Purpose: RG7112 is a small-molecule MDM2 antagonist. MDM2 is a negative regulator of the tumor suppressor p53 and frequently overexpressed in leukemias. Thus, a phase I study of RG7112 in patients with hematologic malignancies was conducted.

Experimental Design: Primary study objectives included determination of the dose and safety profile of RG7112. Secondary objectives included evaluation of pharmacokinetics; pharmacodynamics, such as *TP53*-mutation status and *MDM2* expression; and preliminary clinical activity. Patients were divided into two cohorts: Stratum A [relapsed/refractory acute myeloid leukemia (AML; except acute promyelocytic leukemia), acute lymphoblastic leukemia, and chronic myelogenous leukemia] and Stratum B (relapsed/refractory chronic lymphocytic leukemia/small cell lymphocytic leukemia; CLL/sCLL). Some Stratum A patients were treated at the MTD to assess clinical activity.

Results: RG7112 was administered to 116 patients (96 patients in Stratum A and 20 patients in Stratum B). All patients experi-

enced at least 1 adverse event, and 3 dose-limiting toxicities were reported. Pharmacokinetic analysis indicated that twice-daily dosing enhanced daily exposure. Antileukemia activity was observed in the 30 patients with AML assessed at the MTD, including 5 patients who met International Working Group (IWG) criteria for response. Exploratory analysis revealed *TP53* mutations in 14% of Stratum A patients and in 40% of Stratum B patients. Two patients with *TP53* mutations exhibited clinical activity. p53 target genes were induced only in *TP53* wild-type leukemic cells. Baseline expression levels of *MDM2* correlated positively with clinical response.

Conclusions: RG7112 demonstrated clinical activity against relapsed/refractory AML and CLL/sCLL. *MDM2* inhibition resulted in p53 stabilization and transcriptional activation of p53-target genes. We provide proof-of-concept that *MDM2* inhibition restores p53 function and generates clinical responses in hematologic malignancies. *Clin Cancer Res*; 22(4); 868–76. ©2015 AACR.

Introduction

The tumor suppressor p53 plays a pivotal role in preventing cancer development. p53 is a transcription factor activated by cellular stress that regulates multiple downstream targets implicated in cell-cycle control, apoptosis, DNA repair, and senescence (1). Inactivation of p53 occurs frequently in cancer cells (2),

leading to uncontrolled proliferation and escape from apoptosis. In non-stressed cells, the level of p53 is tightly controlled by murine double minute 2 homologue (*MDM2*). *MDM2* is a key negative regulator of p53 that directly binds the transactivation domain to inhibit its activity (3). Moreover, *MDM2* targets p53 for proteasomal degradation through its E3 ubiquitin-ligase activity (3, 4). Blocking the p53-*MDM2* interaction may overcome the oncogenic consequences of *MDM2* overproduction as well as the pathways associated with inhibiting functional p53, thus restoring p53 tumor-suppressor function (5).

RG7112 is a potent and selective antagonist of the p53-*MDM2* interaction that belongs to the Nutlin family of compounds (6–8). RG7112 binds the p53 pocket on the surface of *MDM2* and mimics the interaction of three amino acid residues (Phe¹⁹, Trp²³, and Leu²⁶) that are critical for binding of *MDM2* to p53, hence effectively preventing their interaction (9). Treatment of cultured human cancer cells harboring wild-type p53 with RG7112 leads to stabilization and accumulation of p53, blocks cell-cycle progression in the G₁ and G₂ phases, and induces apoptosis (6). In addition, preclinical leukemia models have demonstrated sensitivity to RG7112 with rapid induction of apoptosis (6). Moreover, overexpression of *MDM2* occurs frequently in human leukemias (10). We previously demonstrated sensitivity to apoptosis *in vitro* in acute myeloid leukemia (AML; ref. 11) and chronic lymphocytic leukemia (CLL) upon exposure

¹The University of Texas MD Anderson Cancer Center, Houston, Texas. ²The University of Texas Health Science Center at San Antonio, San Antonio, Texas. ³Princess Margaret Cancer Centre, Toronto, Ontario, Canada. ⁴McGill University, Montreal, Quebec, Canada. ⁵Cancer Institute of New Jersey/UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey. ⁶City of Hope National Medical Center, Los Angeles, California. ⁷St. James's Institute of Oncology, Leeds, United Kingdom. ⁸University of Bologna, Bologna, Italy. ⁹Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom. ¹⁰University of Oxford, Oxford, United Kingdom. ¹¹Roche Innovation Center New York, New York. ¹²FORMA Therapeutics, Boston, Massachusetts. ¹³Roche Innovative Center Penzberg, Penzberg, Germany.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

Corresponding Author: Michael Andreeff, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Unit 448, Houston, TX 77030. Phone: 713-792-7261; Fax: 713-794-1903; E-mail: mandreeff@mdanderson.org

doi: 10.1158/1078-0432.CCR-15-0481

©2015 American Association for Cancer Research.

Translational Relevance

The tumor suppressor p53 is frequently dysregulated in cancer. One of the mechanisms by which p53 activity can be restored is by inhibiting the interaction with its negative regulator, MDM2, which is frequently overexpressed in leukemias. Here, we report the results of a multicenter phase I proof-of-concept trial of RG7112, a small-molecule antagonist of MDM2. We demonstrate clinical activity in patients with relapsed/refractory leukemias, such as AML, ALL, CML, and CLL, in which baseline MDM2 levels positively correlated with clinical response. Moreover, we show that inhibition of MDM2 activity can stabilize p53, activate p53-target genes, and induce apoptosis, indicating that p53 tumor-suppressor function can be restored. However, *TP53* status did not define response to RG7112. The results support further investigation of an MDM2 antagonist in hematologic malignancies.

to Nutlin-3a (12), the first MDM2 antagonist (11). Here, we present the phase I, open-label, dose-escalation trial of RG7112 in patients with relapsed/refractory AML, acute lymphocytic leukemia (ALL), chronic myelogenous leukemia-blast crisis (CML-BC), and chronic lymphocytic leukemia/small cell lymphocytic leukemia (CLL/sCLL).

Patients and Methods

The primary objectives of this phase I study (NCT00623870) were to determine the MTD, the dose-limiting toxicities (DLT), and the safety profile of RG7112. The secondary objectives included evaluation of pharmacokinetic (PK) parameters and pharmacodynamic markers for the original crystalline and an alternative amorphous formulation of the compound and assessment of preliminary clinical activity.

Patients and study design

NO21279 was a dose-escalation design with two strata: (A) acute leukemias, including AML, ALL, and CML-BC (excluding acute promyelocytic leukemia; APL); and (B) chronic leukemias, including CLL and sCLL (Supplementary Table S1). All patients were previously treated with relapsed and/or refractory disease and were not considered to be candidates for standard therapies. The trial implemented standard inclusion/exclusion criteria (see Supplementary Methods). Patients signed informed consent, and the study was conducted in accordance with the principles of the "Declaration of Helsinki" and Good Clinical Practice.

RG7112 is a CYP3A4 substrate and inhibitor. Patient did not receive other drugs from these classes where feasible (see Supplementary Methods).

Dosing

The starting dose for the first cohort in both strata was 20 mg/m² of the crystalline formulation of RG7112, administered orally once-daily (QD). Dose escalation within each stratum initially used the crystalline formulation and an accelerated dose-titration design until grade 2 toxicity was demonstrated. This protocol was followed by escalation using a standard 3 + 3 design. RG7112 was administered with or without food, dependent on the specific version of the protocol. There was no inpatient dose escalation.

PK data from a concomitant bioavailability and food-effect study using alternative formulations of RG7112 (NP25299) suggested that an amorphous formulation of RG7112 may improve the variability of exposure seen with the crystalline formulation of the compound used in this study (13). These data also indicated that administering RG7112 with a high-fat, high-calorie meal improved exposure and variability (13). Therefore, after starting this study, the protocol was amended to include the evaluation of an alternative, amorphous formulation of RG7112 in Stratum A patients and to evaluate the effect of dosing with a high-fat/high-calorie meal.

RG7112 was dosed orally for 10 days (initially for PK purposes, one single dose was added on day 3 in cycle 1) followed by 18 days of rest in a 28-day cycle (Supplementary Table S1). Dose escalations and consideration of DLTs and MTDs were conducted independently for each stratum. In addition, the dosing schedule was changed to twice-daily (BID) to improve absorption and gastrointestinal (GI) tolerance at doses greater than 1,000 mg/m². When BID dosing was implemented, the single dose on day 3 was removed. For purposes of PK evaluation in patients receiving BID dosing, only one morning dose was given on day 10 in cycle 1. For the crystalline formulation, doses of 20 to 1,920 mg/m²/day RG7112 were administered either as a single dose on day 3 (followed by a 72-hour washout) then QD for 10 days (day 1–10) or BID for 10 days (no day 3 dosing). When dosing began with the amorphous escalation in Stratum A, the dose was changed to a BID (1,000 mg or 1,500 mg) flat dose for convenience. Another 28 Stratum A patients were treated at the MTD in the efficacy extension, or tail portion, of the study to explore early signals of efficacy. *TP53* status was analyzed in blood samples for all patients and in bone marrow samples for patients in the amorphous escalation and the tail. At least 16 patients with wild-type p53 AML or ALL were accrued into the efficacy extension cohort as defined by the protocol (Supplementary Table S1).

Assessments

The study was conducted at 12 centers: five in the United States, three in the United Kingdom, two in Canada, and two in Italy. During screening, patients were assessed by bone marrow aspirates, biopsies and hematology assessments to confirm leukemia status. For Stratum B patients, imaging was performed for staging per investigator discretion. Adverse events (AE), physical examinations, and blood work were assessed approximately weekly for the first three 28-day cycles and at the beginning and end of treatment dosing in cycle 4 and beyond. Patients were also examined for QTc elongation.

Safety

AEs were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 3.0 (14). Monitoring of AEs continued for up to 28 days after the last dose of a study drug had been administered or until any ongoing event resolved or stabilized. DLTs were assessed during the first treatment cycle (see Supplementary Methods).

Efficacy analysis

In Stratum A patients, efficacy by a hematology assessment and bone marrow assessment (performed at investigator's discretion after cycle 2) every 28 days before the start of each cycle was evaluated according to the International Working Group criteria (15). Patients were scored as having a complete response (CR) and

CR without full platelet recovery (pCR) or a partial response (PR). Although not defined in the protocol, the morphologic leukemia-free state (MLFS), or clearance of bone marrow blasts without recovery of normal peripheral counts, was also assessed as a category of partial response (PR).

Tumor responses in patients in Stratum B were evaluated according to NCI-Working Group criteria (16) every 28 days before the start of each subsequent cycle. Imaging for response assessment was performed at the discretion of the investigator.

Pharmacokinetic analysis

Plasma PK assessments were conducted for all patients during the first cycle of treatment on the first and last day of dosing immediately before dosing, and at multiple post dose time points for measurements of RG7112 concentrations using a validated LC/MS-MS method. Bone marrow PK assessments for RG7112 were performed for all patients before the start of dosing in cycle 1 and 2 and before dosing on the last dosing day (day 10) in cycle 1. Bone marrow samples for RG7112 were assayed using a method modified from the plasma PK assay.

The following PK parameters were estimated using standard noncompartmental methods: AUC_{inf} , the area under the plasma concentration-time curve from 0 to infinity; AUC_T , the area under the plasma concentration-time curve over one dosing interval; C_{max} , maximum observed plasma concentration; t_{max} , time to reach maximum plasma concentration; and $t_{1/2}$, terminal half-life, computed as $(\ln 2)/k_{el}$, where k_{el} - apparent elimination rate, computed as the magnitude of the slope from the log-linear regression of the apparent terminal elimination phase of the plasma concentration-time curve.

Pharmacodynamic analysis

Pharmacodynamic samples were collected before and after administration of RG7112 to measure MIC-1 (macrophage inhibitor cytokine-1) protein in serum using ELISA and reported as percent of change from baseline due to large interpatient variability in baseline MIC-1 levels.

Biomarker analysis

In patients that received the crystalline formulation of RG7112, analysis of *TP53* mutations was performed on Miltenyi magnetic-activated cell sorting (MACS; CD33 or CD34 antibody for selection of AML and CD19 for ALL/CLL)-separated blood. In patients from the amorphous escalation and tail groups, analysis of *TP53* mutations was obtained from BD Vacutainer Cell Preparation Tubes (CPT)-separated blood and MACS-separated bone marrow. This analysis was performed by Caris Life Sciences using the PCR-based *TP53* test. This test reports single nucleotide substitutions or deletions in exons 2–11 and their splice sites (17).

Exploratory analysis of mRNA levels for a panel of 24 direct and indirect p53 target genes (Supplementary Table S2) was evaluated in all patients by qRT-PCR from pre- and post-dose MACS-separated blood and bone marrow using Applied Biosciences TaqMan Low-Density Array (TLDA) cards, in which all samples from each patient were analyzed concurrently [i.e., at 0, 6, 24, 48, 240 (day 10), 246 (day 10 + 6 hours) and 264 (day 11) hours].

MDM2-mRNA concentrations were assessed in amorphous escalation and tail by qRT-PCR with 50-ng total RNA from blood samples collected into Paxgene tubes and MACS-isolated leukemia cells from bone marrow. TaqMan (Invitrogen) probes were designed to detect *MDM2* mRNA and the reference mRNA,

encoding β -glucuronidase, simultaneously using two different fluorescent reporters. Relative gene expression was calculated based on ΔC_t of *MDM2* to housekeeping gene.

In selected samples with sufficient available cells, immunoblot analysis for BBC3 and TP53 was performed, and induction of apoptosis was determined by flow cytometry of annexin V and propidium iodide as described previously (11).

Statistical analyses

Standard summary statistics were calculated and presented for PK, safety, demography, and biomarker data. Spearman correlation coefficients were calculated for MIC-1 versus AUC_{SS} and for best response in AML patients versus relative gene expression levels of *MDM2*. *P* values were calculated using the null hypothesis of Spearman correlation coefficient = 0. No adjustments were made for multiple comparisons.

For biomarker analysis, the statistical analysis approach was used to analyze the peak fold change over all follow-up time points of each patient. Consequently, average peak fold changes were calculated and reported. Genes with a fold change ≥ 2 and *P* value ≤ 0.01 were considered significantly induced or suppressed.

A linear mixed model was used to analyze mRNA level fold changes of all the follow-up time points relative to the baseline, assuming a linear relationship between fold changes and the logarithms of the assigned dose levels. Fold changes for the same patient over different follow-up time points were assumed to be similar, and their averages were calculated and the average fold changes at the MTD are reported.

Results

A total of 116 patients were enrolled into either Stratum A ($N = 96$) or Stratum B ($N = 20$) of this dose-escalation study, inclusive of efficacy extension testing in the tail cohort. *TP53* testing of the patients in the tail assured that at least 16 patients with wild-type p53 AML were treated (Supplementary Table S3). This population was heavily pretreated having at least one previous cancer therapy regimen: mean (SD) of 3.4 ± 1.98 and 4.7 ± 2.92 for Stratum A and Stratum B, respectively (Supplementary Table S4).

Safety results

All patients enrolled in the study ($N = 116$) were safety evaluable and experienced at least 1 AE, with 81 of 116 patients experiencing grade 3 AEs regardless of attribution and 34 of 116 patients experiencing grade 4 AEs across all cohorts during all cycles of therapy (Table 1 and Supplementary Table S5). Serious adverse events (SAE) were reported in 82 patients across all cohorts. The most frequently reported SAEs (>10% of patients) were febrile neutropenia and pneumonia. Eighteen patients discontinued the study due to an AE, most commonly (>3%) febrile neutropenia (3 patients) and pneumonia (3 patients). Most of the AEs leading to discontinuation were of grade 3 (8 patients) or grade 4 (7 patients) severity; however, 3 patients with grade 2 AEs (nausea, abdominal pain, and atrioventricular block) were also discontinued from the study.

There were 3 DLTs in Stratum A in this study: grade 4 pericarditis in 1 patient and grade 3 nausea in 2 patients. These events were all considered to be related to study treatment. As mentioned previously, a fourth patient in Stratum A experienced laboratory

Table 1. On-study AEs > 10% of total and grade 3 or 4 (all patients)

	Total, n (%)	Grade 3, n (%)	Grade 4, n (%)
Total	116 (100)	81 (70)	34 (29)
Nausea	79 (68)	11 (9)	0
Diarrhea	65 (56)	8 (7)	0
Vomiting	42 (36)	6 (5)	0
Fatigue	34 (29)	5 (4)	0
Abdominal pain	31 (27)	10 (9)	1 (<1)
Febrile neutropenia	31 (27)	22 (19)	3 (3)
Decreased appetite	29 (25)	2 (2)	0
Hypokalemia	25 (22)	11 (9)	3 (3)
Headache	20 (17)	1 (<1)	0
Pneumonia	20 (17)	14 (12)	2 (2)
Pyrexia	18 (16)	5 (4)	0
Epistaxis	15 (13)	2 (2)	0
Hypomagnesemia	15 (13)	2 (2)	0
Anemia	14 (12)	6 (5)	4 (3)
Thrombocytopenia	15 (13)	3 (3)	9 (8)
Hypotension	14 (12)	5 (4)	0
Hypocalcemia	14 (12)	5 (4)	0
Asthenia	13 (11)	1 (<1)	0
Constipation	13 (11)	1 (<1)	0

abnormalities consistent with tumor lysis syndrome, which met DLT laboratory criteria, and the protocol was subsequently amended. There were no clinically relevant imbalances in AEs between Stratum A cohorts treated with a QD or BID dosing regimen. The MTD of 1,500 mg BID in Stratum A was based on GI tolerability, and the severity of the AEs was related to exposure. The same proportion of patients treated at the MTD also experienced AEs leading to study discontinuation as those enrolled in Stratum A overall. For Stratum B patients, escalation was halted without DLTs due to excessive pill burden, moderate exposure levels, and limited clinical activity. The highest dose level tested was 1,920 mg/m² BID. Review of QTcF data revealed no evidence of dose or exposure effects after treatment with RG7112.

Thirty-one deaths occurred on study, none of which were considered to be related to the study treatment. The majority of deaths in the study (17/31) were due to disease progression, an additional five were due to underlying disease. Twenty-seven of these 31 deaths occurred in the Stratum A, 14 of which occurred at the MTD. Other primary causes of death reported in Stratum A included common complications of relapse leukemia including pneumonia ($n = 1$), septic shock ($n = 1$), cerebral hemorrhage ($n = 1$), subdural hemorrhage ($n = 1$), acute renal failure ($n = 1$), clostridium difficile colitis ($n = 1$), and fungal infection ($n = 1$). There were four deaths on study in Stratum B, including 2 patients with disease progression and 1 patient with pneumonia and pulmonary sepsis. The fourth patient died due to hemorrhagic stroke related to ongoing thrombocytopenia.

Efficacy dose escalation/extension results

Stratum A. During dose escalation, evidence of clinical antileukemia activity was evident, with a reduction in peripheral blast counts. Most patients had return of leukemia before initiating a second cycle. One patient with AML in the dose-escalation cohort obtained CRp and continued on therapy for six cycles before relapsing.

Forty-three Stratum A patients were treated at the MTD dose 1,500 mg BID (including dose escalation and extension). Of these, 5 patients died before hematologic malignancy evaluation

(day 28) and could not be assessed. An additional 5 patients withdrew early due to a variety of reasons (e.g., AE, hospice decision). In total, 33 patients treated at the MTD dose were evaluable for hematologic malignancy response (Supplementary Table S3). In this evaluable group, 30 patients had a diagnosis of AML, 2 patients obtained CR, and 1 patient obtained CRp for a CR rate of 10% (3/30). Two patients obtained PRs, one of which was considered MLFS without peripheral recovery, for a marrow clearance rate of 13% (4/30). All responses occurred within the first cycle of therapy. Nine patients had stable disease. Sixteen patients progressed during the first cycle of treatment including two before complete dosing. The 2 CR patients were able to bridge to allogeneic transplant, with one engrafting with a maintained remission. There were 3 patients with diagnosis of ALL; 2 had stable disease and 1 progressed on study.

Clinical activity, including CRs, occurred in patients who were primary refractory to 1 or 2 cytarabine-containing chemotherapy regimens. RG7112 also demonstrated clinical activity in extremely poor prognosis patients with multiple relapses and therapy-related disease (Supplementary Table S3). Most responses were observed after the initial cycle of treatment.

Stratum B. Patients in Stratum B also demonstrated evidence of clinical activity, including decreases in peripheral lymphocyte counts, although most of these were transient. Of the 19 patients assessed, 1 patient with CLL (history of prior Richter's transformation) had PR and continued on treatment for 25 cycles. Fifteen patients had stable disease, ranging from two to six cycles, and 3 patients progressed during the first cycle. Even at the highest doses tested (1,920 mg/m²), exposures in CLL patients were lower than the anticipated therapeutic range, complicating a true assessment of antileukemic activity in this heavily pretreated CLL population.

Pharmacokinetic and pharmacodynamic results

We examined the PK of RG7112 in two formulations. The mean PK profiles by dose following drug administration at steady-state (day 10) are presented in Supplementary Data S5. PK parameters are summarized in Supplementary Fig. S1. The dose-exposure (C_{max} , C_{trough} , and AUC) relationship was approximately linear (Fig. 1A), although there was high interpatient variability (CV approximately 70%). A possible absorption plateau was evident when doses exceeded 810 mg/m². The median half-life ($t_{1/2}$) was 1 to 1.5 days. Accumulation was not apparent. BID dosing enhanced daily exposure (Supplementary Fig. S1).

For patients treated at MTD, a single-dose regimen of 1,500 mg BID for 10 days of an amorphous formulation yielded an average AUC₂₄ of 175,529 ng/h/mL ($N = 30$ evaluable for PK, CV 47%) on day 10. The predicted minimum target exposure for efficacy based on animal models was an AUC₂₄ of 200,000 ng/h/mL. Of the patients treated at the MTD, only 11 out of 30 (37%) appeared to have adequate exposure. In AML patients, the patient's best response correlated with steady-state AUC (Spearman correlation coefficient = -0.47 and $P = 0.0001$). AML patients treated at the MTD that showed clinical activity (CR, CRp, PR, and SD) had higher median steady-state AUC than patients that progressed, 221,691 ng/h/mL versus 113,885 ng/h/mL, respectively (Supplementary Tables S6 and S7).

The bone marrow level of RG7112 changed as a function of plasma concentration (Fig. 1B) or dose (data not shown) and was approximately 50% of the blood level at high doses/exposures. Serum levels of MIC-1, a secreted protein that is strongly induced

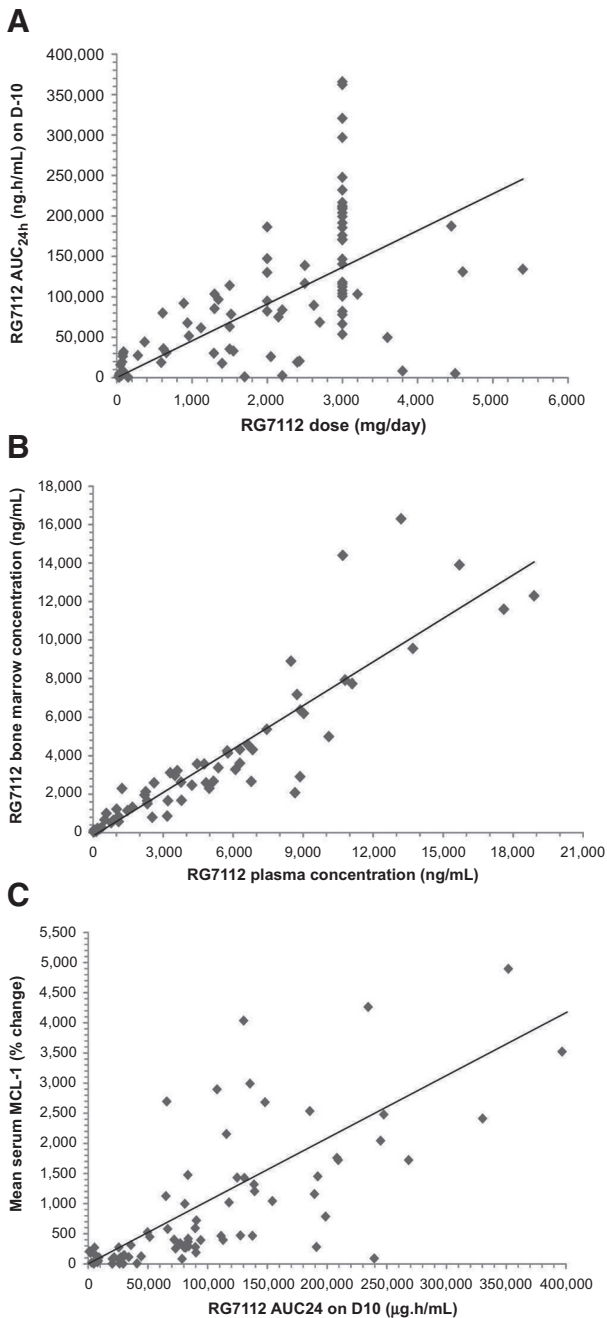


Figure 1. RG7112 pharmacokinetics and pharmacodynamics data. A, correlation between RG7112 dose (mg/day) and exposure (AUC₂₄ hours; ng/h/mL) for Stratum A and Stratum B on day 10. The diamonds represent individual patients. B, correlation between RG7112 levels in bone marrow (ng/mL) and plasma concentration (ng/mL). The diamonds represent individual patients. C, correlation between mean serum MIC-1 levels (% change) and RG7112 plasma levels (AUC₂₄; µg/h/mL) on day 10. The diamonds represent individual patients. MIC-1, macrophage inhibitory cytokine-1; AUC, area under curve; D, day.

by activated p53 (18), were used to assess pharmacodynamic effects of RG7112. Daily dosing yielded higher MIC-1 levels on the last day of dosing, which was partially due to an accumu-

lation of MIC-1 (median $t_{1/2}$ = 46 hours). Analysis of patients on 10-day consecutive dosing of 20 to 2,430 mg/m²/day (given as 1,215 mg/m² BID) showed that the minimum level of RG7112 required for p53 induction was 320 mg/m²/day. MIC-1 levels were elevated at RG7112 plasma concentrations of >2 µg/mL or AUC₂₄ > 50,000 ng/h/mL, highly correlated with exposure (AUC₂₄) at steady state (Fig. 1C). MIC-1 levels in serum (from both tumor and normal cellular sources) did not correlate with clinical activity.

Biomarker results

TP53 mutational analysis identified *TP53* mutations in 19 of 96 patients tested, with more mutations detected in Stratum B patients 40% (6 of 15) than in Stratum A patients 16% (13 of 81; Supplementary Table S8). Each mutation was only detected once, and 2 patients had 2 different mutations. There were 2 patients whose blood and bone marrow results were discordant with mutation in the bone marrow and wild-type in the blood. This difference is likely related to malignant cell prevalence discrepancy between bone marrow and blood, as one patient had no peripheral blasts and the other patient had low peripheral blasts.

Most patients with mutant *TP53* failed to show evidence of response. Two AML patients with *TP53* mutations demonstrated clinical activity by decreased peripheral blast counts, but none had sustained clinical improvement. Patient 8421, with an R175H mutation, had 43% peripheral blasts on day 1, and after receiving nine doses, the peripheral blasts of this patient decreased to 0% on day 22. This patient also had tumor lysis syndrome. Patient 8635 showed a decrease in peripheral blasts from 42% on day 1 to 14% on day 28. Patient 9604 with sCLL/CLL and prior history of transformation to large-cell lymphoma (Richter's transformation), which was not evident at the time of treatment with RG7112, had a 2-bp splice deletion in *TP53*. The patient began treatment in cohort 2 and continued for 25 cycles (over 2 years) with stable disease by clinical exam and PET CT. Although not part of the study procedures, a repeated p53 analysis upon progression demonstrated the same 2-bp deletion in *TP53* and no additional *TP53* abnormalities.

The release of p53 from the p53-MDM2 complex also activates MDM2 expression. Relative MDM2 gene expression levels analyzed by RT-PCR were significantly increased after treatment ($P < 0.001$). In blood, the MDM2 relative median gene expression levels increased 1.9-fold from baseline at day 2 (24 hours after dose) and 2.8-fold on day 10 (the last day of dosing). In bone marrow, the MDM2 relative median gene expression levels increased 2.0-fold on day 10.

The increase in MDM2 expression levels at day 10 of cycle 1 in the blood ($n = 34$) of Stratum A patients correlated with exposure (Fig. 2A; Pearson correlation coefficient = 0.57 and $P = 0.0005$). A correlation was also seen with bone marrow ($n = 16$) on day 10 (Spearman correlation coefficient = 0.59 and $P = 0.02$). There was a trend on day 2 (24 hours post-dose,) for increase in MDM2 expression levels to correlated with the p53-activation biomarker MIC-1 in the blood, albeit with lower significance than with exposure (Fig. 2B, Pearson correlation coefficient = 0.29 and $P = 0.11$).

Table 2 shows the baseline *MDM2* relative gene expression level in blood separated by response. In the limited number of samples tested, the best response in AML patients showed a trend with the relative mean gene expression levels of *MDM2* mRNA in blood at baseline. The best response also correlated ($P = 0.03$) with

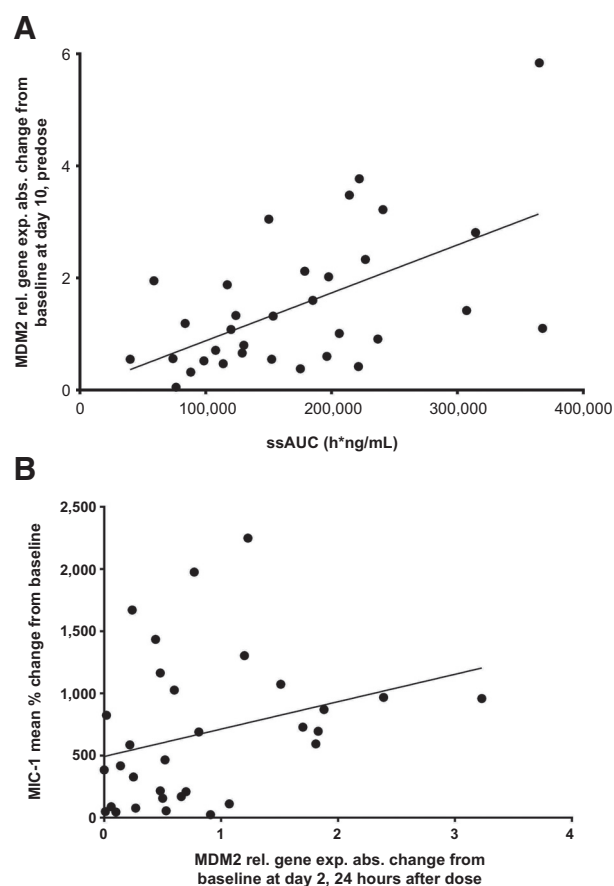


Figure 2.

Increased drug exposure to RG7112 correlates with increased *MDM2* mRNA levels. A, scatter plot of the absolute change from baseline of *MDM2* relative gene expression levels at day 10 predose of cycle 1 in the blood versus AUC_{ss} ($h \cdot ng/mL$) of Stratum A patients. The circles represent individual patients. AUC_{ss} , area under curve at steady state; *MDM2*, murine double minute 2. B, scatter plot of the absolute change from baseline of *MDM2* relative gene expression levels at day 2 24 hours post-dose in the blood versus MIC-1 of Stratum A patients. The circles represent individual patients. MIC-1, macrophage inhibitory cytokine-1.

the absolute change in *MDM2* mRNA relative mean gene expression in blood on day 10. Biomarker analyses were for exploratory purposes only. No adjustment was made for multiple comparisons.

Induction of p53, p53 targets, and apoptosis

MDM2 inhibition is expected to increase cellular p53 levels, activate p53-target genes, and induce apoptosis in leukemia cells (11, 12, 19). The statistical analysis for the peak fold change over all the follow-up time points of each patient was assessed using patients who were assigned to a dose of 810 mg BID or higher

(Fig. 3). Among these patients, a dose higher than 810 mg BID did not change gene expression significantly. As shown in Fig. 3A, 10 genes were significantly induced (≥ 2 -fold induction above baseline and $P \leq 0.01$) in response to RG7112 treatment, all of which are known p53 targets. The cell-cycle regulator *CDKN1A* and the proapoptotic BH3 protein *BBC3/PUMA* were strongly (>5 -fold) induced genes. Because *MDM2* inhibitors, such as RG7112, act by inhibiting p53 degradation but not p53 transcription, the mRNA levels of *TP53* and housekeeping *GAPDH* were not significantly affected by treatment, as expected. Importantly, these 10 genes were induced only in p53 wild-type, and not in p53-mutant, samples (Fig. 3B), consistent with specificity for RG7112 as an inducer of p53. The modest induction of *CDKN1A* (maximal 3-fold) may suggest a cell-cycle effect in these cells (Fig. 3B).

Next, we investigated time-course changes of these 10 induced genes. As shown in Fig. 3B, gene induction occurred as early as 6 hours of treatment and reached a peak after 10 days of therapy (240–264 hours). For the purpose of biomarker analysis of only p53-target genes, clinical activity was defined as 50% reduction in blasts. The strongest induction of p53-target genes was observed in leukemic cells with wild-type p53 for the p53-target genes *BAX*, *BBC3 (PUMA)*, *FDXR*, *MDM2*, and *ZMAT3* (Fig. 3B). Activation of these genes occurred within 6 hours of treatment (Fig. 3B). The death domain-carrying and apoptosis-inducing genes, *FAS* and *TNFRSF10B*, in addition to the cell-cycle-related, p53-target gene *CDKN1A*, peaked within 10 to 11 days (Fig. 3A). Another p53-target gene, *TP53NP1*, was activated at 10 to 11 days. Patients who showed signs of clinical activity versus nonresponders with wild-type p53 differentially expressed increased levels of mRNA for PMA-induced protein 1 (*PMAIP1*; 2.3x; $P = 0.0047$), which binds to *MCL-1* and promotes activation of caspases and apoptosis. Other cell-cycle- or apoptosis-related genes, such as *XPO1* or *APAF1*, were not affected. Table 3 shows the list of genes induced in patients with wild-type p53 treated at MTD (1,500 mg/m^2 BID, $n = 20$), with serial samples available. With the exception of three genes (i.e., *PERP*, *FAS*, and *TPINP1*), the list of genes is shown in Fig. 3.

Induction of *PUMA* and *TP53*, followed by apoptosis, was documented in the limited samples available by immunoblot and flow-cytometric analysis. Supplementary Figure S2 shows induction of *PUMA* and apoptosis in a patient with CLL whose white blood count decreased by $>50\%$.

Discussion

In this phase I study of RG7112, an *MDM2* antagonist, we have demonstrated proof-of-concept in AML, with activation of p53 target genes and complete remissions in extremely poor prognosis, relapsed/refractory patients. Activity was also seen in patients with CLL/sCLL. Responses in patients with acute leukemia allowed normal recovery of peripheral counts and bridging to allogeneic transplantation. While active, this oral compound had significant GI toxicity including nausea, vomiting, and diarrhea,

Table 2. Baseline relative *MDM2* gene expression by best hematologic response in the blood of AML patients

	CR (N = 3)	CRp (N = 2)	PR (N = 3)	SD (N = 21)	PD (N = 40)	MS (N = 15)	Overall (N = 84)
N	2	1	2	10	17	9	41
Mean	1.66	0.73	0.75	0.8	0.54	0.64	0.7

Abbreviations: CR, complete response; CRp, complete response without full platelet recovery; PR, partial response; SD, stable disease; PD, progressive disease; MS, missing or assessment not done; *MDM2*, murine double mutant 2; AML, acute myelogenous leukemia.

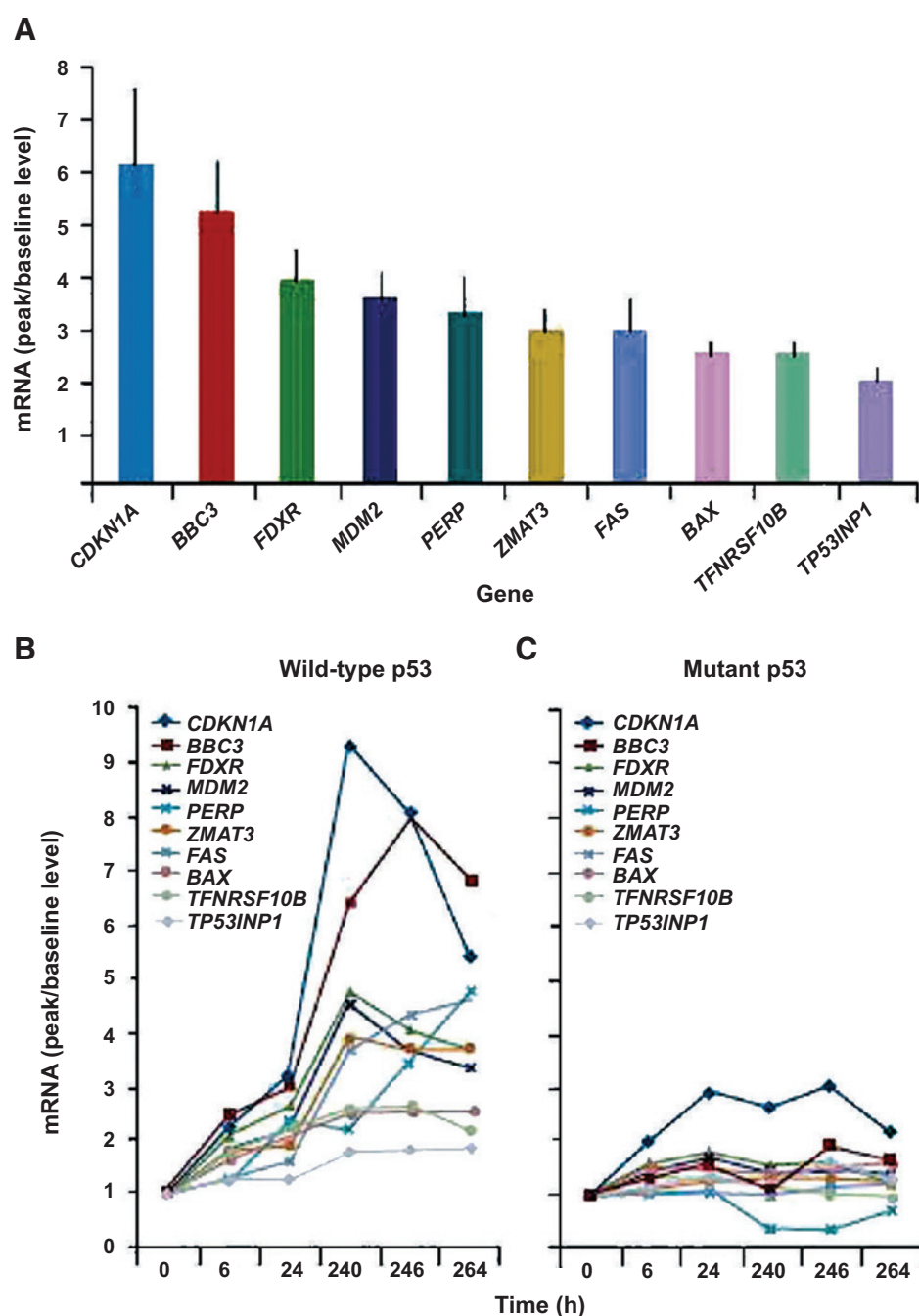


Figure 3. RG7112 induces p53 target gene expression in circulating leukemia blasts. A, mRNA expression levels (peak relative to baseline expression) of p53-target genes significantly induced in leukemia samples. B, expression of p53-target genes in p53 wild-type samples (left) and p53-mutant samples. Gene induction occurred in a wild-type p53 manner. No genes were significantly induced in p53-mutant samples.

Table 3. mRNA fold changes (average over all time points) in p53-target genes in wild-type p53 leukemia samples at MTD

Gene	Fold change	SE	P
BAX	1.85	0.15	<0.0001
BBC3	3.67	0.60	<0.001
CDKN1A	3.95	0.83	0.0005
FDXR	2.63	0.35	<0.0001
MDM2	2.37	0.23	<0.0001
TNFRSF10B	1.82	0.19	<0.0001
ZMAT3	2.09	0.24	<0.0001

which required prophylaxis and treatment. The MTD was based on GI tolerance. Variability in exposure at the MTD limited some patients from achieving target therapeutic exposures, which is apparent from AUC₂₄ data for the overall response group [CR, PR, SD, or HI (hematologic improvement)] at the MTD versus those patients who progressed during the initial cycle.

No molecular marker(s) has yet been identified, which predicts response to an MDM2 antagonist. Data collected for chromosomal (e.g., translocations) and molecular (e.g., *FLT3* mutations) leukemic prognostic markers did not demonstrate any predictive

value to differentiate between the responders versus nonresponders in this phase I study. This class of agents is only predicted to be effective in patients with at least 1 wild-type *TP53* allele. However, wild-type *TP53* status alone does not predict response to MDM2 antagonists. Evidence of activity in patients with p53 mutation could be due to multiple clones being present, (only some of which are mutant), mutation in a single allele with retention of the wild-type allele, or that certain *TP53* mutations may still have functional p53 activity and the ability to respond. A marker of functional *TP53* activity that can be correlated with response in patients with wild-type, and possibly mutant, protein needs to be identified. Elevation of MDM2 in leukemia cells at baseline correlated with clinical response in this small population of patients, as previously reported by us *in vitro* (12), but it was not sufficient as a single predictive marker of sensitivity to treatment with an MDM2 antagonist. A 4-gene molecular signature that includes MDM2 is currently being investigated as a potential predictor of sensitivity (20). In this study, a total of 10, among 24, genes were significantly changed in a wild-type p53 manner. All of the 10 genes have been shown to be p53 targets, and results provided a proof-of-concept that MDM2 inhibition by RG7112 activates p53 *in vivo* and induces a broad panel of p53 target genes in leukemia cells. The target gene induced most effectively, *CDKN1A* (p21), is a critical cell-cycle regulator, which may counteract induction of apoptosis (21, 22).

An important question for the long-term use of MDM2 antagonists in cancer patients is whether an MDM2 antagonist may select for a mutant population of tumor cells. Although this study was not designed to answer that question, one CLL/sLL patient with a 2-bp *TP53* deletion maintained stable disease for more than 2 years. Repeat analysis of *TP53*-mutational status in the tumor at progression showed the identical mutation and no new mutations.

Although RG7112 has demonstrated activity as a monotherapy in relapsed/refractory AML, the future development of MDM2 antagonists for treatment of leukemia will require combination therapies. Results of a study combining this compound with cytarabine (both low and intermediate doses) have been presented (23). Evidence for activity of combinations with non-chemotherapeutic targeted agents has been developed, including combinations (24–35).

MDM2 is critical for normal hematopoiesis, and treatment with an MDM2 antagonist leads to significant marrow suppression of both leukemic and normal progenitors. As expected, complications of neutropenia and thrombocytopenia, including sepsis and hemorrhage, were seen in this heavily pretreated AML population. Monotherapy efficacy was also evident, including patients who achieved complete remissions with hematopoietic recovery and ability to bridge to transplant, warranting further evaluation of MDM2 antagonist therapy of acute leukemias.

The RG7112 molecule has several disadvantages, including a high dose required for efficacy leading to GI intolerance and

variability of exposure at the MTD. To address these issues, development of the Nutlin MDM2 antagonists will continue with a more potent compound RG7388, which has an identical p53-binding properties and mechanism of action, and is now in phase I clinical development in AML (35).

Disclosure of Potential Conflicts of Interest

S. Assouline reports receiving a commercial research grant from and is a consultant/advisory board member for Roche Canada. M.W. Drummond reports receiving speakers bureau honoraria from Celgene, Novartis, Sanofi, and Shire, and is a consultant/advisory board member for Novartis. M. Kirschbaum is a consultant/advisory board member for Spectrum. P. Bridge has ownership interest (including patents) in Hoffmann–La Roche. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M. Andreeff, M. Kirschbaum, G. Chen, P. Bridge, S. Middleton, M. Reckner, R. Rueger, J. Zhi, G. Nichols
Development of methodology: M. Andreeff, M. Kirschbaum, G. Chen, S. Blotner, P. Bridge, R. Rueger, J. Zhi, G. Nichols, K. Kojima
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Andreeff, K.R. Kelly, K. Yee, S. Assouline, R. Strair, L. Popplewell, D. Bowen, G. Martinelli, M.W. Drummond, P. Vyas, M. Kirschbaum, S.P. Iyer, V. Ruvolo, G. Chen, G. Nichols, K. Kojima
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Andreeff, K.R. Kelly, M. Kirschbaum, S.P. Iyer, V. Ruvolo, G.M. Noguera González, X. Huang, G. Chen, S. Blotner, P. Bridge, L. Jukofsky, R. Rueger, J. Zhi, G. Nichols, K. Kojima
Writing, review, and/or revision of the manuscript: M. Andreeff, K.R. Kelly, K. Yee, S. Assouline, L. Popplewell, D. Bowen, M.W. Drummond, P. Vyas, M. Kirschbaum, S.P. Iyer, G.M. Noguera González, X. Huang, G. Chen, B. Graves, S. Blotner, P. Bridge, L. Jukofsky, S. Middleton, M. Reckner, R. Rueger, J. Zhi, G. Nichols, K. Kojima
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Andreeff, P. Vyas, M. Kirschbaum, V. Ruvolo, L. Jukofsky, M. Reckner, R. Rueger, G. Nichols
Study supervision: M. Andreeff, M.W. Drummond, M. Kirschbaum, S.P. Iyer, P. Bridge, G. Nichols
Other (focus of contribution is on clinical safety data): P. Bridge

Acknowledgments

The authors thank the patients who participated in this study and their families. They also thank Hagop M. Kantarjian, Susan O'Brien, Gautam Borthakur, Theresa McQueen, Mary A. Kelly, Lyubovir Vassilev, as well as the nurses, investigators, and others on local study teams for their contributions to this trial.

Grant Support

This work was supported in part by Hoffmann–La Roche, Inc. and by grants from the NIH (P01 CA49639 and CA16672; to M. Andreeff) and by the Paul and Mary Haas Chair in Genetics (to M. Andreeff). Support for third-party writing assistance for this article was provided by Hoffmann–La Roche, Inc.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 27, 2015; revised August 24, 2015; accepted September 21, 2015; published OnlineFirst October 12, 2015.

References

- Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature* 2000; 408:307–10.
- Xu-Monette ZY, Medeiros LJ, Li Y, Orlowski RZ, Andreeff M, Bueso-Ramos CE, et al. Dysfunction of the TP53 tumor suppressor gene in lymphoid malignancies. *Blood* 2012;119:3668–83.
- Marine JC, Lozano G. Mdm2-mediated ubiquitylation: p53 and beyond. *Cell Death Differ* 2010;17:93–102.
- Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 1992;69:1237–45.

5. Chene P. Inhibiting the p53-MDM2 interaction: an important target for cancer therapy. *Nat Rev Cancer* 2003;3:102-9.
6. Tovar C, Graves B, Packman K, Filipovic Z, Higgins B, Xia M, et al. MDM2 small-molecule antagonist RG7112 activates p53 signaling and regresses human tumors in preclinical cancer models. *Cancer Res* 2013;73:2587-97.
7. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, et al. *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 2004;303:844-8.
8. Vu B, Wovkulich P, Pizzolato G, Lovey A, Ding Q, Jiang N, et al. Discovery of RG7112: a small-molecule MDM2 inhibitor in clinical development. *ACS Med Chem Lett* 2013;4:466-9.
9. Fry DC, Wartchow C, Graves B, Janson C, Lukacs C, Kammlott U, et al. Deconstruction of a nutlin: dissecting the binding determinants of a potent protein-protein interaction inhibitor. *ACS Med Chem Lett* 2013;4:660-5.
10. Bueso-Ramos CE, Yang Y, deLeon E, McCown P, Stass SA, Albitar M. The human MDM-2 oncogene is overexpressed in leukemias. *Blood* 1993;82:2617-23.
11. Kojima K, Konopleva M, Samudio IJ, Shikami M, Cabreira-Hansen M, McQueen T, et al. MDM2 antagonists induce p53-dependent apoptosis in AML: implications for leukemia therapy. *Blood* 2005;106:3150-9.
12. Kojima K, Konopleva M, McQueen T, O'Brien S, Plunkett W, Andreeff M. Mdm2 inhibitor Nutlin-3a induces p53-mediated apoptosis by transcription-dependent and transcription-independent mechanisms and may overcome Atm-mediated resistance to fludarabine in chronic lymphocytic leukemia. *Blood* 2006;108:993-1000.
13. Patnaik A, Tocher A, Beeram M, Nemunaitis J, Weiss G, Nichols G. Clinical pharmacology of RG7112, an MDM2 antagonist, in patients with advanced solid tumors [abstract]. In Proceedings: AACR 104th Annual Meeting 2013; Apr 6-10, 2013; Washington, DC. Abstract nr LB-201.
14. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol* 2003;13:176-81.
15. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood* 2008;111:5446-56.
16. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *J Clin Oncol* 2003;21:4642-9.
17. Dicker F, Herholz H, Schnittger S, Nakao A, Patten N, Wu L, et al. The detection of TP53 mutations in chronic lymphocytic leukemia independently predicts rapid disease progression and is highly correlated with a complex aberrant karyotype. *Leukemia* 2009;23:117-24.
18. Yang H, Filipovic Z, Brown D, Breit SN, Vassilev LT. Macrophage inhibitory cytokine-1: a novel biomarker for p53 pathway activation. *Mol Cancer Ther* 2003;2:1023-9.
19. Kojima K, Konopleva M, Samudio IJ, Schober WD, Bornmann WG, Andreeff M. Concomitant inhibition of MDM2 and Bcl-2 protein function synergistically induce mitochondrial apoptosis in AML. *Cell Cycle* 2006;5:2778-86.
20. Zhong H, Chen G, Jukofsky L, Geho D, Han SW, Birzele F, et al. MDM2 antagonist clinical response association with a gene expression signature in acute myeloid leukaemia. *Br J Haematol* 2015;171:432-5.
21. Drakos E, Thomaidis A, Medeiros LJ, Li J, Leventaki V, Konopleva M, et al. Inhibition of p53-murine double minute 2 interaction by nutlin-3A stabilizes p53 and induces cell cycle arrest and apoptosis in Hodgkin lymphoma. *Clin Cancer Res* 2007;13:3380-7.
22. Kobayashi T, Consoli U, Andreeff M, Shiku H, Deisseroth AB, Zhang W. Activation of p21WAF1/Cip1 expression by a temperature-sensitive mutant of human p53 does not lead to apoptosis. *Oncogene* 1995;11:2311-6.
23. Yee K, Martinelli G, Assouline S, Kasner M, Vey N, Kelly KB. Phase 1b study of the MDM2 antagonist RG7112 in combination with 2 doses/schedules of cytarabine. *Blood* 2013;122:498.
24. Carter BZ, Mak DH, Schober WD, Koller E, Pinilla C, Vassilev LT, et al. Simultaneous activation of p53 and inhibition of XIAP enhance the activation of apoptosis signaling pathways in AML. *Blood* 2010;115:306-14.
25. Drakos E, Atsaves V, Li J, Leventaki V, Andreeff M, Medeiros LJ, et al. Stabilization and activation of p53 downregulates mTOR signaling through AMPK in mantle cell lymphoma. *Leukemia* 2009;23:784-90.
26. Kojima K, Konopleva M, Samudio IJ, Ruvolo V, Andreeff M. Mitogen-activated protein kinase inhibition enhances nuclear proapoptotic function of p53 in acute myelogenous leukemia cells. *Cancer Res* 2007;67:3210-9.
27. Kojima K, Konopleva M, Tsao T, Andreeff M, Ishida H, Shiotsu Y, et al. Selective FLT3 inhibitor FI-700 neutralizes Mcl-1 and enhances p53-mediated apoptosis in AML cells with activating mutations of FLT3 through Mcl-1/Noxa axis. *Leukemia* 2010;24:33-43.
28. Kojima K, Konopleva M, Tsao T, Nakakuma H, Andreeff M. Concomitant inhibition of Mdm2-p53 interaction and Aurora kinases activates the p53-dependent postmitotic checkpoints and synergistically induces p53-mediated mitochondrial apoptosis along with reduced endoreduplication in acute myelogenous leukemia. *Blood* 2008;112:2886-95.
29. Kojima K, Kornblau SM, Ruvolo V, Dilip A, Duvvuri S, Davis RE, et al. Prognostic impact and targeting of CRM1 in acute myeloid leukemia. *Blood* 2013;121:4166-74.
30. Kojima K, McQueen T, Chen Y, Jacamo R, Konopleva M, Shinojima N, et al. p53 activation of mesenchymal stromal cells partially abrogates microenvironment-mediated resistance to FLT3 inhibition in AML through HIF-1alpha-mediated down-regulation of CXCL12. *Blood* 2011;118:4431-9.
31. Kojima K, Shimanuki M, Shikami M, Andreeff M, Nakakuma H. Cyclin-dependent kinase 1 inhibitor RO-3306 enhances p53-mediated Bax activation and mitochondrial apoptosis in AML. *Cancer Sci* 2009;100:1128-36.
32. Kojima K, Shimanuki M, Shikami M, Samudio IJ, Ruvolo V, Corn P, et al. The dual PI3 kinase/mTOR inhibitor PI-103 prevents p53 induction by Mdm2 inhibition but enhances p53-mediated mitochondrial apoptosis in p53 wild-type AML. *Leukemia* 2008;22:1728-36.
33. Thompson T, Andreeff M, Studzinski GP, Vassilev LT. 1,25-dihydroxyvitamin D3 enhances the apoptotic activity of MDM2 antagonist nutlin-3a in acute myeloid leukemia cells expressing wild-type p53. *Mol Cancer Ther* 2010;9:1158-68.
34. Yoshimura M, Ishizawa J, Ruvolo V, Dilip A, Quintas-Cardama A, McDonnell TJ, et al. Induction of p53-mediated transcription and apoptosis by exportin-1 (XPO1) inhibition in mantle cell lymphoma. *Cancer Sci* 2014;105:795-801.
35. Ding Q, Zhang Z, Liu JJ, Jiang N, Zhang J, Ross TM, et al. Discovery of RG7388, a potent and selective p53-MDM2 inhibitor in clinical development. *J Med Chem* 2013;56:5979-83.