Preclinical Development

The Novel Tryptamine Derivative JNJ-26854165 Induces Wild-Type p53- and E2F1-Mediated Apoptosis in Acute Myeloid and Lymphoid Leukemias

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Abstract

The development of small-molecule activators of p53 is currently focused on malignancies containing a wild-type p53 genotype, which is present in most leukemias. JNJ-26854165 is one such p53-activating agent, but its mechanism of action remains to be elucidated. Here, we report the effects of JNJ-26854165 in acute leukemias. JNJ-26854165 treatment induced p53-mediated apoptosis in acute leukemia cells with wild-type p53, in which p53 rapidly drives transcription-independent apoptosis followed by activation of a transcription-dependent pathway. JNJ-26854165 accelerated the proteasome-mediated degradation of p21 and antagonized the transcriptional induction of p21 by p53. Interestingly, JNJ-26854165 induced S-phase delay and upregulated E2F1 expression in p53 mutant cells, resulting in apoptosis preferentially of S-phase cells. E2F1 knockdown blocked apoptosis induced by JNJ-26854165 in p53 mutant cells. Apoptotic activity of JNJ-26854165 against primary acute leukemia cells was maintained in leukemia/stroma cocultures, unlike doxorubicin, which has reduced cytotoxicity in coculture systems. JNJ-26854165 synergizes with 1-β-arabinofuranosylcytosine or doxorubicin to induce p53-mediated apoptosis. Our data suggest that JNJ-26854165 may provide a novel therapeutic approach for the treatment of acute leukemias. The presence of p53-independent apoptotic activity in addition to p53-mediated apoptosis induction, if operational in vivo, may prevent the selection of p53 mutant subclones during therapy. Mol Cancer Ther; 9(9); OF1–13. ©2010 AACR.

Introduction

p53-induced apoptosis plays an important role in preventing cancer development (1). p53 is the most frequently inactivated protein in human cancer, and more than 50% of all solid tumors carry TP53 mutations (2). TP53 mutations rarely occur in leukemias, but inactivation of wild-type p53 frequently occurs through binding to its principal cellular regulator HDM2 (3, 4). HDM2 binds p53 at the transactivation domain of p53 and blocks its ability to activate transcription, serves as a ubiquitin ligase that promotes p53 degradation, and mediates the nuclear export of p53 (5). HDM2 has been found to be overexpressed in approximately 50% of acute leukemias (3, 4), a process that can actively enhance tumorigenicity and resistance to apoptosis.

There is evidence that transformed cells are more sensitive to p53-induced apoptosis than are their normal counterparts, leading to the suggestion that activation of p53 may cause tumor-specific cell killing (6, 7). Activation or restoration of p53 response therefore becomes an attractive therapeutic goal (6–11). Small molecule inhibitors have been described that disrupt HDM2-p53 binding, which liberates p53 from its inhibitor and enables p53 activation (9–11). One of these compounds, Nutlin-3, binds HDM2 in the p53-binding pocket, effectively dislodging p53 from HDM2 and inducing p53 response, which inhibits growth and induces p53-mediated apoptosis in leukemias (4, 11–14). Based on preclinical data, phase I trials are ongoing to determine the maximally tolerated dose and activity of the HDM2 inhibitor R7112 (Nutlin-3 analog) in leukemia (NCT00623870) and solid tumor (NCT00559533) patients. Another such compound is MI-63, which is quite similar in its mechanism of action (10). JNJ-26854165 is a novel p53-activating tryptamine derivative that was initially thought to act as a HDM2 ubiquitin ligase antagonist (15, 16). Preclinical data have shown potent antiproliferative activity in various p53 wild-type and mutant tumor models, implying p53-independent activities. JNJ-26854165 entered a phase I study to determine the safety and dose in patients with advanced-stage or refractory solid tumors (NCT00676910).

Note: Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

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In this study, we investigated the potential therapeutic utility of p53 activation by JNJ-26854165 in acute leukemias. We found that (a) JNJ-26854165 treatment induces p53-mediated apoptosis in acute leukemia cells, (b) p53 rapidly drives transcription-independent apoptosis before the activation of the transcription-dependent pathway in the cells, (c) JNJ-26854165 induces S-phase delay and E2F1-mediated apoptosis in p53 mutant cells, and (d) JNJ-26854165 synergizes with 1-β-arabinofuranosylcytosine (AraC) or doxorubicin to induce p53-mediated apoptosis.

Materials and Methods

Reagents

JNJ-26854165 was provided by Ortho Biotech Oncology Research and Development. Nutlin-3 and pifithrin-μ were purchased from Cayman Chemical, and pifithrin-α from Tocris Bioscience. In some experiments, cells were cultured with 70 μmol/L cycloheximide, 10 μmol/L MG132, or 100 μmol/L Z-VD-FMK (Axxora), which were added to the cells 1 hour before drug administration. Cells were synchronized at the G1-S boundary by 18-hour treatment medium supplemented with 10% FCS at a density of 5 × 10^5 cell/mL. Normal bone marrows were obtained after January 20, 2018. © 2010 American Association for Cancer Research. mct.aacrjournals.org Downloaded from mct.aacrjournals.org on January 20, 2018. © 2010 American Association for Cancer Research.
JNJ-26854165 Induces Apoptosis in Acute Leukemias

The following antibodies were used: rabbit polyclonal anti-p53 (Santa Cruz Biotechnology), mouse monoclonal anti-p53 (DO-1, Santa Cruz Biotechnology), mouse monoclonal anti-p21 (EMD Biosciences), mouse monoclonal anti-HDM2 (Santa Cruz Biotechnology), rabbit polyclonal anti-HDMX (Bethyl Laboratories), mouse monoclonal anti-Noxa (EMD Biosciences), rabbit polyclonal anti-Puma (EMD Biosciences), mouse monoclonal anti-E2F1 (Santa Cruz Biotechnology), mouse monoclonal anti-p73 (Santa Cruz Biotechnology), rabbit polyclonal anti-phospho-retinoblastoma (Rb; Ser628; Cell Signaling Technology), mouse monoclonal anti-Rb (Cell Signaling Technology), and mouse monoclonal anti-β-actin (Sigma).

Western blot analysis

Equal amounts of protein lysate were separated by SDS-PAGE (12% gel) for 2 hours at 80 V. Proteins were transferred to Hybond-P membranes (Amersham Biosciences), immunoblotted with appropriate antibodies, and reacted with enhanced chemiluminescence reagent (Amersham Biosciences). Signals were detected by phosphorimager Storm 860 (Molecular Dynamics). An anti-β-actin blot was used in parallel as a loading control. Visualized blots were analyzed by the ImageJ 1.38 software (NIH).

Immunofluorescence and confocal microscopy

Cells were incubated with MitoTracker Red CMXRos (300 nmol/L) for 30 minutes at 37°C, washed twice with PBS, fixed with 2% paraformaldehyde, and permeabilized with ice-cold 100% methanol. The cells were blocked in 5% normal goat serum for 30 minutes, followed by incubation overnight at 4°C with Alexa Fluor 488–conjugated rabbit polyclonal anti-p53 antibodies (300 nmol/L) according to the manufacturer’s instructions (Amaxa Biosystem). To evaluate the transfection efficiency, cells were transfected with the BLOCK-IT Fluorescent-Oligo (Invitrogen); efficiency of transfection was estimated to be approximately 70%, with approximately 90% cell viability. E2F1 and HDM2 expression was respectively downregulated by using the ON-TARGETplus SMART pool L-003259 and L-003279 (Dharmacon). Nonspecific control pool (Dharmacon) served for a negative control. Forty-eight hours posttransfection, E2F1, HDM2, and β-actin expression was analyzed by Western blot analysis.

Statistical analysis

Statistical analysis was done using the two-tailed Student’s t test. Results were considered statistically significant at P <.05. Unless otherwise indicated, average values are expressed as mean ± SD. Synergism, additive effects, and antagonism were assessed, as previously described. The combination index (CI), a numerical description of combination effects, was calculated using the more stringent statistical assumption of mutually nonexclusive modes of action (19). When CI = 1, this equation represents the conservation isobologram and indicates additive effects. CI values <1.0 indicate a more-than-expected additive effect (synergism), whereas CI values >1.0 indicate antagonism between the two drugs.

Results

JNJ-26854165 inhibits cell growth and induces apoptosis in leukemia cell lines

We first examined the effect of JNJ-26854165 on the growth of cultured leukemia cell lines. The results showed that JNJ-26854165 exhibited antiproliferative activity in a variety of leukemia cell lines (Supplementary Table S1). The IC_{50} values at 72 hours, defined as the concentration of JNJ-26854165 causing 50% growth inhibition, were in general lower in p53 wild-type cells than in p53 mutant cell lines, although the differences were small when compared with Nutlin-3. To clarify if the antiproliferative activity of JNJ-26854165 was associated with induction of apoptosis, Annexin V–positive fractions and cellular DNA content were determined. Treatment of p53 wild-type cells with JNJ-26854165 induced apoptosis in a time- and dose dependent manner, as evidenced by high Annexin V positivity (Fig. 1A and Supplementary Fig. S1A) and increased sub-G1 DNA content (Supplementary Fig. S1B). JNJ-26854165 induced...
Figure 1. Functional p53 expression is required for maximal induction of apoptosis by JNJ-26854165. A, cells were treated with 5 μmol/L JNJ-26854165 for the indicated times, and the Annexin V–positive fractions were measured by flow cytometry. B and C, parental and lentivirally transduced OCI-AML3 cells [virus encoding either scrambled shRNA (shC) or p53-specific shRNA (shp53)] were incubated with the indicated concentrations of JNJ-26854165 for 48 hours, and CMXRos retention (B) and the number of viable cells (C) were assessed. Results are expressed as the percentage of the numbers of viable cells in an untreated group. *, significance at $P < 0.05$. p53 expression was determined by Western blot analysis in untreated or γ-irradiated cells 5 hours after 6 Gy irradiation (RT). D, cells were incubated with 5 μmol/L JNJ-26854165 (JNJ-165) for 48 hours and stained for DNA content. Cell cycle distribution was analyzed using ModFit LT software. Results are representative of three independent experiments.
delayed apoptosis in p53 mutant cells, which was seen after 72 or 96 hours of exposure (Fig. 1A and Supplementary Fig. S1B). The delayed apoptosis occurred in a dose-dependent manner (Supplementary Fig. S1C). Consistent with these findings, 48-hour treatment with JNJ-26854165 resulted in a significant increase of trypan blue–positive cells in wild-type p53 cells but only minimally affected the viability of p53 mutant cells (figure not shown). These results suggest that JNJ-26854165 induces early apoptosis in p53 wild-type cells whereas it inhibits cellular proliferation followed by delayed apoptosis in the absence of functional p53.

To further define the observed cell growth inhibition and cell death induced by JNJ-26854165, we investigated the effect of JNJ-26854165 in OCI-AML-3 cells infected with lentivirus encoding either scrambled short hairpin RNA (shRNA) or p53-specific shRNA (20). We have reported that knockdown of p53 rendered OCI-AML-3 cells resistant to Nutlin-induced apoptosis. As shown in Fig. 1B, p53 knockdown cells were less sensitive to JNJ-26854165 than were parental or scrambled shRNA-expressing OCI-AML-3 cells resistant to Nutlin-induced apoptosis. In accordance with the time course of protein expression, transcriptional activation of p53-regulated genes was not seen at 6 hours but was clearly detectable at 18 hours after exposure to JNJ-26854165 in OCI-AML-3 (Fig. 2D) and MOLM-13 (Supplementary Fig. S2) cells. The p53-regulated gene expression patterns after JNJ-26854165 treatment were similar to those after Nutlin-3. JNJ-26854165 treatment did not alter p53 mRNA levels, suggesting that JNJ-26854165 has little effect on p53 synthesis. The time course of protein and transcript expression was similar in the two other studied p53 wild-type cell lines, NALM-6 and REH. These findings suggest that JNJ-26854165 accumulates p53 and thereby evokes p53-mediated responses.

Wild-type p53 rapidly translocates to mitochondria in response to JNJ-26854165

JNJ-26854165 induced apoptosis after as early as 6 hours in p53 wild-type cells (Fig. 3A) when transcriptional activation of p53-regulated genes was present (Fig. 2D and Supplementary Fig. S2). Such early induction of apoptosis was not seen in p53 mutant cells (Figs. 1A and 3A). It has been reported that a cytoplasmic pool of p53 can induce apoptosis through a transcription-independent mechanism (21). Pifithrin-α specifically inhibits p53 binding to mitochondria by reducing its affinity to the antiapoptotic proteins Bcl-xL and Bcl-2 but has no effect on p53-dependent transactivation (22). As shown in Fig. 3B, 6-hour cotreatment with pifithrin-α protected p53 wild-type cells partially from JNJ-26854165-induced apoptosis. Because 24-hour exposure to pifithrin-α resulted in massive apoptosis, it remains unknown if this partial protection implies the presence of pathways distinct from p53-mediated and transcription-independent mechanism in JNJ-26854165-induced apoptosis or the interference of pifithrin-α toxicity. We also used pifithrin-γ, a transcriptional inhibitor of p53, to investigate the possible relevance of transcription-dependent p53 function.
in the early response of cells to JNJ-26854165. OCI-AML-3, MOLM-13, NALM-6, and REH cells were treated for 6 hours with 30 μmol/L JNJ-26854165 and 10 μmol/L pifithrin-α individually and in combination, and the Annexin V-positive fractions were measured by flow cytometry. Pifithrin-α did not reduce JNJ-26854165-induced phosphatidylserine externalization (data not shown). Transcriptional activation of target genes of p53 occurs in the nucleus, whereas mitochondrion-targeted p53 induces transcription-independent apoptosis (22). We determined the intracellular localization of p53 in OCI-AML-3 cells using confocal microscopy. A small amount of p53 was diffusely distributed in untreated cells (Fig. 3C). p53 did not specifically colocalize with the mitochondrial marker MitoTracker Red CMXRos [Pearson’s correlation coefficient, r = 0.02 ± 0.06 (mean ± SE), n = 54). After an 8-hour treatment with 10 μmol/L JNJ-26854165, individual cells showed either cytoplasmic (31%), cytoplasmic and nuclear (48%), or nuclear (21%) accumulation of p53. Independent of cellular p53 localization, approximately 70% of cells showed punctated cytoplasmic signals of p53. p53 frequently colocalized with the mitochondrial marker MitoTracker Red CMXRos (r = 0.42 ± 0.08, n = 48). p53 did not show positive or negative colocalization with DAPI (r = -0.04 ± 0.07), arguing against the idea that accelerated nuclear export of p53 contributed to mitochondrial accumulation of p53.

**JNJ-26854165 reduces the rate of DNA synthesis**

Increased S-phase percentage in p53-deficient cells was associated with a decrease in cell number, suggesting that JNJ-26854165 slowed or halted DNA synthesis. To determine if JNJ-26854165 affects DNA synthesis, leukemia...
cell lines were treated with 5 μmol/L JNJ-26854165 for 24 hours and were pulse-labeled for 1 hour with BrdUrd. Although JNJ-26854165 treatment increased the percentage of cells incorporating BrdUrd, we observed that pulse labeling with BrdUrd of JNJ-26854165–treated cells resulted in lower levels of BrdUrd than control cells, as indicated by decreased mean fluorescence intensity (Fig. 4A and B). The delayed DNA synthesis was p53 independent. In p53 wild-type leukemia cells in which JNJ-26854165 induced p53-dependent apoptosis, the small fraction of cells that escaped into S-phase had significantly lower mean cellular BrdUrd intensity than control cells (Fig. 4A and B). To further assess the effects of JNJ-26854165 on S-phase progression, p53 mutant U937 cells were synchronized at the G1-S boundary with hydroxyurea and released into S-phase in the absence or presence of JNJ-26854165. As shown in Fig. 4C, S-phase progression was delayed over a 12-hour period.

**JNJ-26854165 induces E2F1-mediated apoptosis in p53 mutant cells**

It has been reported that HDM2 stabilizes E2F1 protein in a p53-independent manner, resulting in E2F1-mediated apoptosis (23, 24). To investigate the molecular events that contribute to p53-independent apoptosis induced by JNJ-26854165 treatment, p53 mutant cells were treated with 5 μmol/L JNJ-26854165 for 48 hours and HDM2-related protein levels were investigated (Fig. 5A). Increased levels of HDM2 and E2F1 upon JNJ-26854165 treatment were detected in three of four cell lines examined. p73 and Noxa levels were increased in two lines. Quantitative real-time PCR (TLDA) was done in four p53 mutant leukemia cell lines including U937, K562, P12-ICHIKAWA, and PF-382 cells (Fig. 5B). The transcriptional activation of p53-regulated genes was poorly detected except for CDKN1A/p21 (induced in three of four cell lines) or TP53INP1/SIP (induced in all four cell lines).
Figure 4. JNJ-26854165 delays S-phase progression independent of p53. A and B, quantitation of BrdUrd incorporation in S-phase cells. Cells were pulse-labeled with BrdUrd for 1 hour and analyzed by flow cytometry for DNA content and BrdUrd incorporation. S-phase cells incorporating BrdUrd were gated and analyzed to determine the mean fluorescence intensity of BrdUrd staining relative to that of G1-phase cells (MFIS_G1). Changes in MFIS_G1 are reported as the ratio between the MFIS_G1 of treated cells compared with the MFIS_G1 of untreated control cells (MFIS_G1/control MFIS_G1). DNA, propidium iodide. C, U937 cells were treated with 1 mmol/L hydroxyurea (HU) for 18 hours, inducing a block at the G1-S boundary. Release into DMSO for the indicated time shows S-phase progression over a 12-hour period. Release into JNJ-26854165 shows a delay in S-phase progression.
CDKN1A/p21 and TP53INP1/SIP have been reported to be E2F1 target genes (25, 26). PMAIP1/Nova and BC3/Puma are also E2F1 target genes (27), and modest (2- to 6-fold mRNA) induction was detected in two of four cell lines. To elucidate if JNJ-26854165 induces E2F1-mediated apoptosis, E2F1 levels were acutely reduced in U937 cells using small interfering RNA (siRNA). E2F1 siRNA led to a significant (∼65%) inhibition of E2F1 expression relative to cells transfected with a control siRNA, and did not interfere with β-actin synthesis (Fig. 5C). Knockdown of E2F1 in U937 cells significantly affected their susceptibility to apoptosis induced by JNJ-26854165 (35.7 ± 4.1% versus 22.4 ± 2.6% sub-G₁ cells; P = 0.0088), suggesting that E2F1 activation plays a role in apoptosis induction.
by JNJ-26854165. Consistent with this hypothesis, JNJ-26854165 induced p53-independent apoptosis predominantly in S-phase cells (Fig. 5D). Knockdown of E2F1 did not affect the increase in S-phase percentage upon JNJ-26854165 treatment (not shown), implying that p53-independent apoptosis may not be directly associated with S-phase delay. Next, HDM2 levels were similarly reduced using siRNA to test the hypothesis that HDM2 expression modulates E2F1. HDM2 siRNA led to a significant (~75%) inhibition of HDM2 expression relative to cells transfected with control siRNA 48 hours after transfection (Supplementary Fig. S3). However, E2F1 levels did not change significantly. Knockdown of HDM2 did not affect the susceptibility to undergo apoptosis or the ability to induce p53-mediated apoptosis in primary acute leukemia cells.
S-phase delay upon JNJ-26854165 treatment. These data suggest that JNJ-26854165 may upregulate E2F1 in an HDM2-independent manner.

**JNJ-26854165 induces apoptosis in primary acute leukemia cells**

We examined the apoptotic effect of JNJ-26854165 on primary cells from 16 patients with AML and 5 patients with ALL. The percentages of spontaneous apoptosis as determined by Annexin V positivity in the series were 25.1 ± 2.8% (mean ± SE; range, 4.8–38.8%) in AML at 72 hours and 28.9 ± 3.4% (20.4–38.6%) in ALL at 48 hours. Fifteen AML samples and all five ALL samples were sensitive to Nutlin-induced apoptosis, suggesting wild-type p53 status. The relation between p53 status and apoptotic sensitivity to Nutlins has already been established. The remaining one AML sample showed a minimal (3%) increase in the proportion of Annexin V–binding cells upon 10 μmol/L Nutlin-3 treatment, and had a missense mutation S215G that occurred in the DNA-binding domain. The sample showed only a moderate increase in the proportion of Annexin V–binding cells upon 10 μmol/L Nutlin-3 treatment (22.1% specific apoptosis). As shown in Fig. 6A, treatment of Nutlinsensitive samples with JNJ-26854165 caused a dose-dependent increase in the percentage of Annexin V–positive cells. We correlated the extent of apoptosis induced by 10 μmol/L JNJ-26854165 with that induced by 5 μmol/L Nutlin-3 in the AML samples. The levels of JNJ-26854165–induced apoptosis directly correlated (r = 0.73; P < 0.05) with those induced by Nutlin-3. p53-related gene expression and protein levels were investigated in two AML samples with >90% blasts and wild-type p53 confirmed by sequencing analysis (Fig. 6B). Increased protein levels of HDM2, p53, and Puma, and transcriptional activation of p53-regulated genes, including CDKN1A/p21, HDM2, BBC3/PUMA, and PMAIP1/NOXA, were detected after 48-hour exposure to JNJ-26854165 in both cases. JNJ-26854165 induced Noxa at the protein level in one sample. p21 protein levels were not increased in either case despite its increased mRNA expression.

**Apoptotic activity of JNJ-26854165 is enhanced by combination with AraC or doxorubicin**

AraC is one of the most active chemotherapeutic agents for the therapy of acute leukemias, and it remains the backbone of induction and consolidation regimens. To determine if JNJ-26854165 treatment in AML or ALL cells might potentiate the effects of AraC, we combined JNJ-26854165 and AraC in acute leukemia cell lines. An interaction study between JNJ-26854165 and AraC showed highly synergistic effects on apoptosis induction in four acute leukemia cell lines with wild-type p53 tested (Supplementary Table S2), with averaged CI values ranging from 0.2 to 0.3. Potentiation effects were not seen in p53 mutant U937, K562, P12-ICHIKAWA, or PF-382 cells (not shown). We then cultured primary cells from three AML and one ALL patients with >90% blasts with JNJ-26854165 and/or AraC, and evaluated apoptosis after 48 hours. The cells had wild-type p53. The averaged CI values ranged from 0.4 to 0.7, confirming the synergistic nature of the JNJ-26854165/AraC interaction in primary leukemia cells (Supplementary Table S2).

Anthracyclines are another class of chemotherapeutic agents commonly used in the treatment of acute leukemias. Similar to the experiments with AraC, cells were treated for 48 hours with JNJ-26854165 and doxorubicin either as individual agents or in combination. The results indicated an additive or slightly synergistic interaction of JNJ-26854165 and doxorubicin on induction of apoptosis (Supplementary Table S3), with averaged CI values ranging from 0.3 to 1.1 (median, 0.9). Potentiation effects were not seen in p53 mutant leukemia cell lines (not shown).

**Apoptotic activity of JNJ-26854165 against acute leukemia cells is relatively maintained in leukemia/stroma cocultures**

Nine primary acute leukemia samples (six AML and three ALL) were cultured alone or cocultured with bone marrow–derived stromal cells from a normal donor. The stromal cells were resistant to 5 μmol/L Nutlin-3, 10 μmol/L JNJ-26854165, or 400 nmol/L doxorubicin, as evidenced by unchanged sub-G1 DNA content after a 96-hour exposure to these agents. The percentages of spontaneous apoptosis as determined by Annexin V positivity in AML series and an ALL sample were 32.6 ± 1.9% (mean ± SE; range, 27.1–39.5%; at 72 hours) and 24.9 ± 5.6% (19.2–36.1%; at 48 hours), respectively. The percentages of cells undergoing apoptosis after exposure to 5 μmol/L Nutlin-3, 10 μmol/L JNJ-26854165, and 400 nmol/L doxorubicin, were 53.3 ± 3.3%, 71.7 ± 5.5%, and 65.1 ± 4.4% in AML, and 73.3 ± 7.2%, 75.7 ± 12.2%, and 87.3 ± 2.7% in ALL, respectively. Coculture of the stromal cells with acute leukemia cells protected the latter from both spontaneous apoptosis and apoptosis induced by Nutlin-3, JNJ-26854165, or doxorubicin (Fig. 6C). However, the protective effect was smaller in Nutlin-3 or JNJ-26854165 than in doxorubicin. Nutlin-3, JNJ-26854165, and doxorubicin are p53 activators, but the mechanisms and off-target effects are different for each compound. Our results may suggest that p53 induction by Nutlin-3 or JNJ-26854165 is more efficient in overcoming stromal cell–mediated leukemia cell resistance than wide-ranging DNA damage responses.

**Discussion**

The identification and characterization of a compound that activates p53 has strong implications for both the development of novel chemotherapeutics and the understanding of the p53 pathway. A majority of leukemias retain wild-type TP53 and have developed mechanisms to disrupt p53 signaling, such as HDM2/HDMX overexpression or CDKN2A/ARF deletions (3, 28, 29). In such cases, use of small-molecule direct activators of p53 may induce sufficient levels of the protein to signal apoptosis.
It has been suggested that restoration of p53 activity utilizing small molecules Nutlins or RITA (reactivation of p53 and induction of tumor cell apoptosis) may be attractive chemotherapeutics in leukemias (4, 14, 30, 31). In this study, we have shown that JNJ-26854165 induces p53-mediated apoptosis in leukemia cells and may be an attractive chemotherapeutic as a single agent or in combination with AraC or anthracyclines. A phase I pharmacokinetic and pharmacodynamic study of JNJ-26854165 in patients with advanced refractory solid tumors showed that after consecutive doses of 300 mg daily, peak plasma concentrations ranged from 2 to 3 µg/mL (6–9 µmol/L), which is well within the dose range used in our studies (15, 32).

Although functional p53 protein contributed to apoptosis induction in leukemia cell lines by JNJ-26854165, p53 might not be essential for long-term cytotoxic effects. E2F1, at least partially, was found to contribute to p53-independent apoptosis induction by JNJ-26854165. Interestingly, the HDM2 antagonist Nutlin-3 has been reported to increase chemotherapy-induced apoptosis in cancer cells lacking functional p53 by activating E2F1 (33, 34). Furthermore, E2F1 transcriptional activity has been reported to be a critical determinant of Nutlin-3–induced apoptosis in human tumor cell lines (35). We think that targeting E2F1 may be exploited in the treatment of tumors without functional p53 and that the E2F1-inducing activity of JNJ-26854165 may be a potential benefit in its development as a cancer therapeutic.

p21, a transcriptional target of p53, has been shown to protect cells from p53-dependent and -independent apoptosis. It is therefore a reasonable strategy to combine p53 activators with agents that inhibit transcriptional induction of p21 and/or degrade p21 (36). Interestingly, unlike Nutlin-3, JNJ-26854165 itself induced proteasome-mediated degradation of p21, antagonizing transcriptional induction of p21 by p53. Although the precise mechanism remains unknown, Nutlin-3, which binds to HDM2 in the p53-binding pocket, has been described to limit the binding of HDM2 to other substrates including p21 (9). Similar to the recently reported scenario in RITA (9), JNJ-26854165 may enhance p53-mediated apoptosis via degradation of p21.

We were surprised to observe that JNJ-26854165 initiates p53-mediated apoptosis before increase in cellular levels of p53 and transcriptional induction of p53 target genes, when a fraction of cytoplasmic p53 was associated with mitochondria. The conventional view of p53-mediated apoptosis has emphasized its role as a transcription factor. However, accumulating evidence indicates that p53 restoration also triggers transcription-independent apoptosis (25, 37, 38). The latter has been reportedly mediated by mitochondrial translocation of cytoplasmic p53, for which p53-dependent transcription is irrelevant (37–40). Our data shown here are compatible with the interpretation that transcription-independent mechanisms are important for p53-mediated induction of apoptosis.

In conclusion, our work suggests that JNJ-26854165 may provide a novel therapeutic tool for the therapy of acute leukemias and may be partially dependent on and independent of p53 mutation status. This latter feature, if operational in vivo, could also delay or prevent the selection of p53 mutant subclones during therapy.

Disclosure of Potential Conflicts of Interest

J. Arts is an employee of Ortho Biotech Oncology Research & Development. M. Andreeff: commercial research grant.

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