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RESEARCH

Targeting MDM2-mediated suppression of p53 with idasanutlin: a promising therapeutic approach for acute lymphoblastic leukemia

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Abstract

Despite available treatments for acute lymphoblastic leukemia (ALL), the disease's high clinical variability necessitates new therapeutic strategies, particularly for patients with high-risk features. The tumor suppressor protein p53, encoded by the *TP53* gene and known as the guardian of the genome, plays a crucial role in preventing tumor development. Over 90% of ALL cases initially harbor wild-type *TP53*. Reactivation of p53, which is encoded from the wild type *TP53* but lost its function for several reasons, is an attractive therapeutic approach in cancer treatment. p53 can be activated in a non-genotoxic manner by targeting its primary repressor, the MDM2 protein. Clinical trials involving MDM2 inhibitors are currently being conducted in a growing body of investigation, reflecting of the interest in incorporating these treatments into cancer treatment strategies. Early-phase clinical trials have demonstrated the promise of idasanutlin (RG7388), one of the developed compounds. It is a second-generation MDM2-p53 binding antagonist with enhanced potency, selectivity, and bioavailability. The aim of this study is to evaluate the efficacy of RG7388 as a therapeutic strategy for ALL and to investigate its potential impact on improving treatment outcomes for high-risk patients. RG7388 potently decreased the viability in five out of six ALL cell lines with diverse *TP53* mutation profiles, whereas only one cell line exhibited high resistance. RG7388 induced a pro-apoptotic gene expression signature with upregulation of p53-target genes involved in the intrinsic and extrinsic pathways of apoptosis. Consequently, RG7388 led to a concentration-dependent increase in caspase-3/7 activity and cleaved poly (ADP-ribose) polymerase. In this research, RG7388 was investigated with pre-clinical methods in ALL cells as a novel treatment strategy. This study suggests further functional research and in-vivo evaluation, and it highlights the prospect of treating p53-functional ALL with MDM2 inhibitors.

Keywords MDM2–p53 antagonists · MDM2 · P53 · Idasanutlin (RG7388) · Acute lymphoblastic leukaemia (ALL) · Targeted therapies

Introduction

Acute lymphoblastic leukemia (ALL) remains a challenging hematologic malignancy characterized by its heterogeneous clinical presentations and treatment outcomes [1]. Despite significant advances in chemotherapy and targeted therapies, a subset of patients, particularly those with high-risk features such as myeloid mutations or Philadelphia chromosome–positive (Ph^+) ALL, continue to face poor prognosis and therapeutic resistance [2, 3]. This clinical landscape underscores the urgent need for novel therapeutic strategies to improve outcomes in ALL.

The *TP53* gene, encoding the tumor suppressor protein p53, plays a pivotal role in safeguarding cellular integrity by regulating key cellular processes including apoptosis, DNA repair, and cell cycle arrest [4]. Loss or dysfunction of p53, observed in a variety of cancers including ALL, can lead to unchecked cell proliferation and survival, contributing to tumor progression. *TP53* mutations are prevalent in about 50% of solid tumors, promoting oncogenic processes by enhancing cellular proliferation and survival [5]. In contrast, *TP53* mutations are relatively uncommon in ALL, occurring in less than 10% of cases at diagnosis and up to 30% at relapse [6, 7]. This rarity is particularly notable in pediatric ALL [7].

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Central to regulating p53 activity is murine double minute 2 (MDM2), an E3 ubiquitin ligase that binds to p53, inhibiting its transcriptional activity and promoting its proteasomal degradation [8]. Overexpression of MDM2, observed in various cancers [9–11], including ALL [12, 13], contributes to p53 inactivation and tumor progression [14]. Targeting the MDM2-p53 interaction with small molecule inhibitors represents a promising therapeutic approach to restore p53 tumor suppressor function in cancers with wild-type *TP53* [15].

Idasanutlin (RG7388) has emerged as a potent and selective MDM2 antagonist designed to disrupt the MDM2-p53 interaction, leading to stabilization and activation of p53 in cancer cells [16, 17]. Preclinical studies have demonstrated that idasanutlin induces apoptosis specifically in *TP53* wild-type cells, while *TP53* mutant cells exhibit resistance, highlighting its therapeutic specificity [18–21]. Mechanistically, idasanutlin-mediated p53 activation results in the upregulation of pro-apoptotic genes involved in both intrinsic (mitochondrial-mediated) and extrinsic (death receptor-mediated) apoptotic pathways [22, 23]. This activation culminates in increased caspase activity and cleavage of poly (ADP-ribose) polymerase (PARP), key markers of apoptotic cell death.

Recent clinical trials have provided encouraging insights into the therapeutic potential of idasanutlin in hematologic malignancies. A Phase I trial evaluating idasanutlin in combination with either standard chemotherapy or venetoclax in relapsed or refractory acute myeloid leukemia (AML) patients demonstrated manageable safety profiles and promising efficacy, with a subset of patients achieving complete responses [24]. Furthermore, clinical trials, whether ongoing or completed but not yet published, are investigating idasanutlin as monotherapy or in combination with other agents in diverse diseases, aiming to validate its clinical benefit and define optimal treatment regimens (ClinicalTrials.gov identifier: NCT02633059 and NCT03287245). The preliminary outcomes of these clinical trials underline idasanutlin's role as a promising therapeutic strategy for *TP53* wild-type ALL, potentially offering a new avenue to improve outcomes in patients with limited treatment options.

Materials and methods

Cell lines and compound

Human ALL cell lines including MOLT-4, RS4;11, Nalm-6, HAL-01, REH, and CCRF-CEM were sourced from authenticated cell line repositories (ATCC or DSMZ) and maintained in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal calf serum and 100 U/mL penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO, USA). Idasanutlin, obtained from Cayman Chemical Company (MI, USA), was dissolved in DMSO (Sigma-Aldrich) to use at a final concentration of 0.5% (v/v) DMSO for experimental use.

Cell viability assay

Cells were plated at a density of 2.5×10^5 cells/mL in 100 µL of culture medium per well of a 96-well plate (Corning) 24 h before treatment with idasanutlin at concentrations ranging from 10 to 3000 nM for 72 h. The XTT Assay Kit (Cayman Chemical Company, MI, USA) was employed to assess growth inhibition relative to a dimethyl sulfoxide (DMSO) control.

Immunoblotting

1×10^6 cells/mL were seeded in 2 mL per well of a 6-well plate (Corning) and subjected to the corresponding manipulation (exposure to idasanutlin). Protein lysates were harvested using 2% SDS lysis buffer at 24 h, heated at 95 °C for 10 min, and sonicated. Protein concentration was measured using a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, UK). Primary antibodies against p53 (DO-7, CST), $p21^{WAF1/CIP1}$ (#12D1, CST), MDM2 (#OP46, Merck Millipore), PARP (#46D11, CST), PUMA (#D30C10, CST), Actin (Sigma) and secondary goat anti-mouse (7076 S, CST)/rabbit (7074 S, CST) horseradish peroxidase-conjugated antibodies were used. All antibodies were diluted in 5% (*w*/*v*) nonfat milk or BSA in TBS-tween20. Proteins were visualized using enhanced chemiluminescence reagents (GE Healthcare).

Caspase 3/7 activity assay

Following treatment with idasanutlin, caspase 3/7 activity was measured as an indicator of apoptotic cell death using the Caspase-Glo® 3/7 Assay Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Real-time reverse transcriptase polymerase chain reaction gene expression analysis 1×10cells/ml were seeded in 1.5 ml per well of a 12-well plate and exposed to RG7388 or DMSO alone for 6 h. Total RNA was extracted from the pellets, using an RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The RNA purity and concentration were determined by measuring the optical density (O.D.) at 260 nm with a spectrophotometer. The A_{260}/A_{280} ratio was used to assess RNA purity. An A_{260}/A_{280} ratio of 1.8–2.1 is indicative of highly purified RNA. Complementary DNA (cDNA) was generated using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems by Thermo Fisher Scientific, 4368814) as described by the manufacturer. qRT-PCR was carried out using SYBR

green RT-PCR master mix (Life Technologies) as per the manufacturer's guidelines using validated cDNA primers [18]. Each sample was analysed in triplicate using GAPDH as a housekeeping control. A no-template-control was used to control for contamination of external DNA in reactions. The mRNA expression of each gene, expressed as Ct values (cycle number to reach critical threshold), was compared with its DMSO-treated matched sample.

Statistical analysis

Data from the repeated experiments were presented as mean ± standard error of the mean (SEM) unless otherwise stated. Statistical tests were carried out using GraphPad Prism 6 software and all *p*-values represent paired or unpaired *t*-tests of at least three independent repeats unless otherwise stated.

Results

ALL cells demonstrate sensitivity to MDM2 inhibition using RG7388

Six ALL cell lines were exposed to increasing concentrations (0 to 3000 nM) of the MDM2 inhibitor RG7388. An XTT assay was used to measure the relative growth of the cells. Figure 1 shows growth inhibition curves for the ALL cell lines treated by RG7388 for 72 h. Five out of six ALL cell lines were sensitive ($IC_{50} \leq 289$ nM) to the MDM2 inhibitor (Fig. 1A-E) compared to the other cell line CCRF-CEM (Fig. 1F). RG7388 showed no significant effect on *TP53* double mutant CCRF-CEM cells even at concentrations up to 3 µM (Fig. 1F and G). Supplementary Table S1 summarises the IC_{50} values of RG7388 and *TP53* gene status in all ALL cell lines used in this study. The REH cell line carrying the heterozygous c.541 C > T mutation in the *TP53* gene (Fig. 1H) had anincreased IC_{50} value compared to other sensitive cells (Nalm-6 $IC_{50} = 82 \pm 6$ nM vs. REH $IC_{50} = 289 \pm 9$ nM; unpaired t-test, $p = 0.0022$). Despite this increase, mutant p53 REH remains within a drug-sensitive range [20], indicating that it still responds to the treatment.

Functional activation of the p53 pathway in wild-type *TP53* ALL cell lines in response to RG7388

Western blot analysis was employed to assess the stabilization of p53 protein and the expression of downstream target genes involved in cell cycle arrest and apoptosis. Following treatment with RG7388, a concentration-dependent stabilization of p53 protein was observed in Nalm-6 and RS4;11 cell lines, which are known to have wild-type *TP53* status. This stabilization was accompanied by an increase in $p21^{WAF1}$ protein levels, a well-established downstream target of p53 involved in cell cycle arrest (Fig. 2A). RG7388 treatment resulted in the upregulation of MDM2 protein levels in a concentration-dependent manner in p53 wild-type cells (Fig. 2B). Conversely, in the *TP53*-mutant cell line CCRF-CEM, RG7388 did not induce stabilization of p53 or upregulation of MDM2 (Fig. 2B). MOLT-4 cells did not show evidence of functional p53 activation (Fig. 2A). Functionally, RG7388-induced p53 activation in Nalm-6 and RS4;11 cells was associated with the induction of apoptotic pathways, as evidenced by the upregulation of pro-apoptotic protein PUMA (p53-upregulated modulator of apoptosis) (Fig. 2B). Additionally, despite the mutation, REH cells exhibited p53 activation and $p21^{WAF1}$ upregulation, highlighting that this specific mutation still allows for drug responsiveness and has clinical significance (Fig. 2A).

Caspase 3/7 activation in response to RG7388 treatment in p53 wild-type ALL cell lines

The assessment of apoptosis induction was further investigated using the caspase 3/7 enzymatic assay, a reliable indicator sensitive to apoptosis [25]. ALL cell lines including Nalm-6, RS4;11, REH, and CCRF-CEM were treated with varying concentrations of RG7388 over 24 and 48 h (Fig. 3). A concentration-dependent increase in caspase 3/7 activity was evident in Nalm-6, RS4;11, and REH cell lines following RG7388 treatment compared to the DMSO control (Fig. 3A-C). Conversely, CCRF-CEM cells, characterized by a double mutant *TP53* status, did not exhibit a significant rise in caspase 3/7 activity even at high concentrations (5 µM) of RG7388, indicating resistance to apoptotic induction mediated by MDM2 inhibition (Fig. 3D). The consistent response between 24-hour and 48-hour treatments suggests that RG7388's apoptotic effect stabilizes within the first 24 h of exposure, with no additional enhancement over prolonged exposure.

Transcriptional activation of p53 target genes by RG7388 in p53 wild-type ALL cell lines

To investigate the hypothesis that RG7388 enhances p53 transcriptional activity in ALL cells treated with MDM2 inhibitors, we assessed mRNA expression levels of candidate genes related to cell cycle arrest and apoptosis using quantitative real-time PCR (qRT-PCR). The study focused on two p53 wild-type (p53WT) cell lines, Nalm-6 and RS4;11, as well as one p53 mutant (p53MUT) cell line, CCRF-CEM. Cells were treated with RG7388 at concentrations of 0.1, 0.3, or 1 µM, along with a DMSO control, for 6 h. Overall, RG7388 treatment induced the expression of candidate genes primarily in p53WT cells (Fig. 4A-B) compared to p53MUT

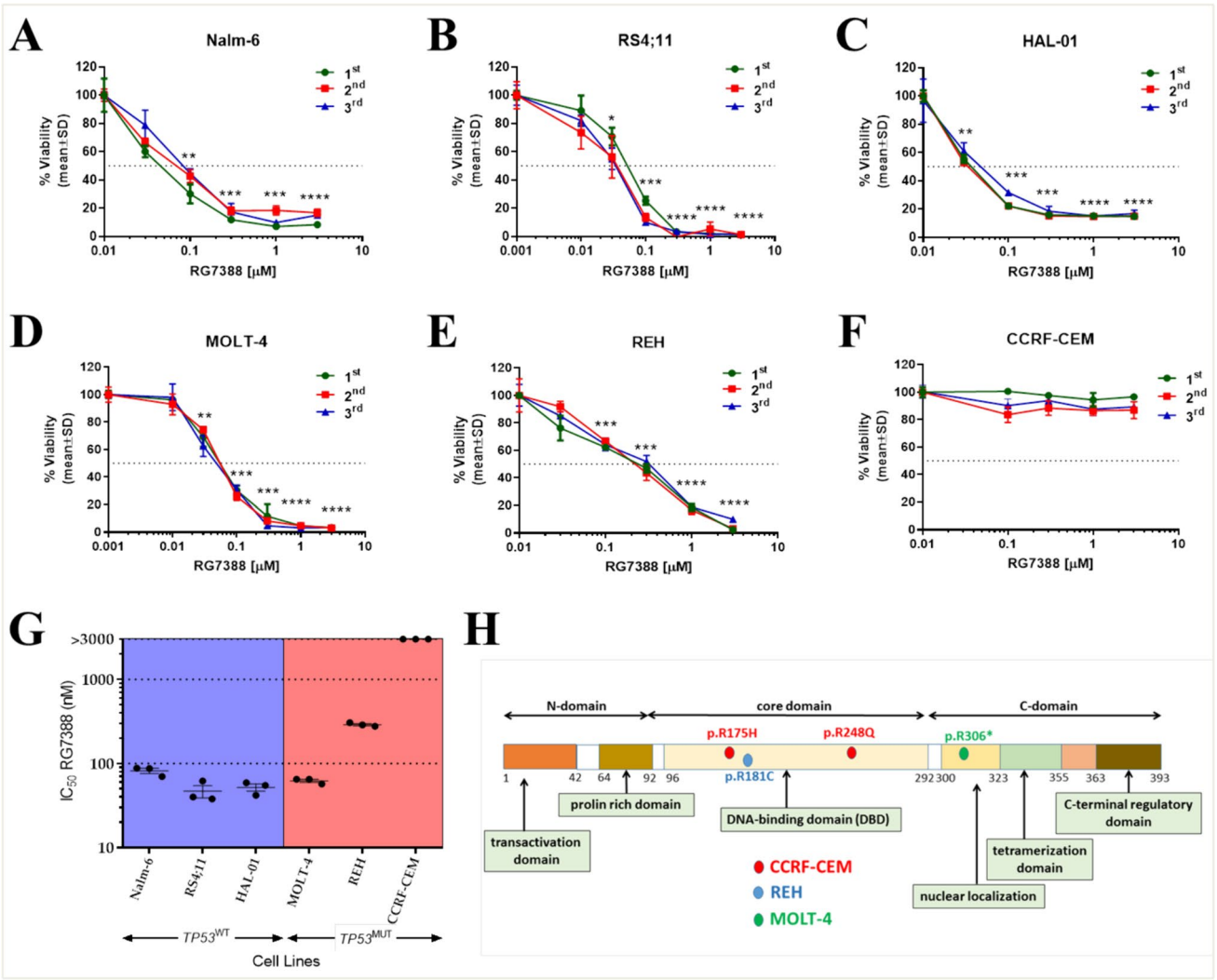

Fig. 1 The concentration-dependent effect of RG7388 on the proliferation of a panel of ALL cell lines. **(A)** Nalm-6, **(B)** RS4;11, **(C)** HAL-01, **(D)** MOLT-4, **(E)** REH, and **(F)** CCRF-CEM. Each cell line shows an independent repeat (e.g. 1st: green, 2nd: red and 3rd: blue). Bars show the mean ± SD. The stars show how significantly the drug induces cell death for each dose compared to DMSO control. Each independent repeat of experiment was averaged within itself and then paired t-test was applied to compare $n = 3$ paired measurements. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. **(G)** Summary IC_{50} values of RG7388 for the panel of ALL lines. The results for *TP53* wild-type ($TP53^{WT}$) and *TP53* mutant ($TP53^{MUT}$) cell lines are shown in blue and red boxes, respectively. Each dot shows an independent repeat. Bars show the mean ± SEM. **(H)** Domain organization of p53 protein. Point mutations of *TP53* mutant ALL cell lines are indicated in different colors (red: CCRF-CEM; blue: REH; green: MOLT-4)

CCRF-CEM cells (Fig. 4C). In Nalm-6 and RS4;11 cells, the fold changes in mRNA expression in response to RG7388 were concentration-dependent (Fig. 4A-B). Specifically, genes associated with cell cycle arrest, such as *CDKN1A* (p21), and the autoregulatory negative feedback loop involving *MDM2*, were significantly upregulated upon RG7388 treatment in p53WT cells (Fig. 4A-B). RG7388 treatment resulted in significant induction of mRNA expression for several pro-apoptotic genes including *BAX*, *FAS*, *PUMA*, *TP53INP1*, and *TNFBSF10B* across all concentrations tested (Fig. 4A-B).

Discussion

This study investigated the efficacy and mechanisms of RG7388, a second-generation MDM2 inhibitor and a member of the Nutlin family, in targeting ALL cells with varying *TP53* gene status. The findings shed light on RG7388's potential as a therapeutic agent and provide insights into its genotype-specific effects on p53 pathway activation and apoptotic induction.

Cell proliferation and viability assay revealed distinct sensitivity profiles among the ALL cell lines to RG7388.

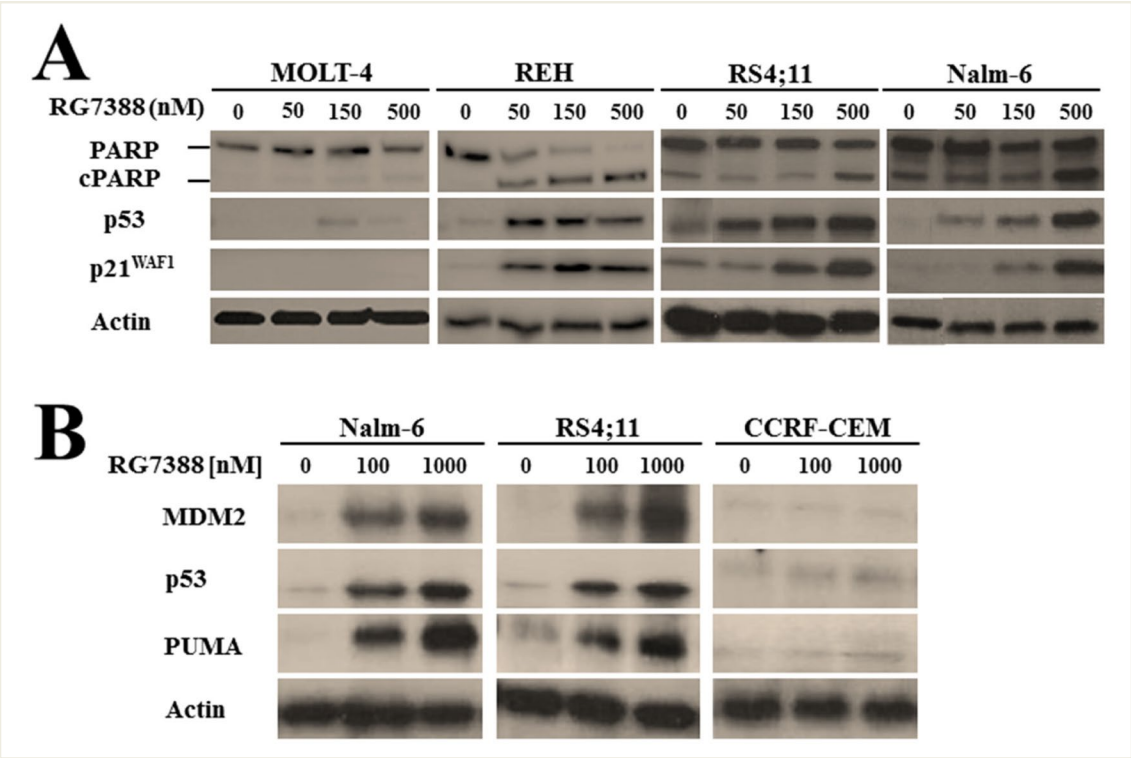

Fig. 2 Functional evaluation of the p53 pathway. RG7388 induced p53 stabilization and upregulation of p53 transcriptional targets, $p21^{WAF1}$**(A)** and MDM2 **(B)**, 24 h after the commencement of treatment in *TP53* wild-type ALL cell lines RS4;11 and Nalm-6 with the indicated doses (nM). No effect was observed on downstream transcriptional targets of p53 in mutant *TP53* cell line CCRF-CEM. An increase in cleaved PARP (cPARP) and induction of PUMA protein were used as markers of apoptosis

Notably, five out of six cell lines demonstrated heightened sensitivity with IC_{50} values $\leq$ 289 nM, indicating potent growth inhibition in response to MDM2 inhibition. In contrast, CCRF-CEM cell line exhibited resistance ($IC_{50} > 3$ μM), suggesting reduced susceptibility to RG7388 treatment.

MOLT-4 cells have a mutation in the *TP53* gene that causes a premature stop codon (p.R306*) (Fig. 1H). This truncates the p53 protein, likely leading to loss of function of the protein. The mutation is located in the nuclear localization region of p53 [26], specifically in exon 8. There was no evidence of functional p53 that could be seen in Western blot experiments (Fig. 2A). The sensitive response to MDM2 suppression in these cells, however, suggests that RG7388 suppresses cell growth through alternative pathways independent of p53. While MDM2 is best known for being the primary negative regulator of p53, a growing body of evidence suggests that MDM2 has a number of functions independent of its role in modulating p53 activation [27, 28].

The REH cell line harbors a *TP53* mutation that results in an arginine to cysteine substitution at position 181 within the p53 protein, located in the DNA-binding domain (DBD) encoded by exon 5 (Fig. 1H). Despite this mutation, REH cells exhibit sensitivity to the MDM2 inhibitor RG7388, albeit with a slightly higher IC_{50} compared to *TP53* wild-type cell lines (Fig. 1G). Western blot analysis reveals that RG7388 stabilizes p53 and increases the expression of its transcriptional target $p21^{WAF1}$ in REH cells (Fig. 2A). This indicates a specific response to the compound despite the presence of the *TP53* mutation, suggesting that even mutated p53 in REH cells retains functional activity that can be targeted by RG7388 [29].

CCRF-CEM cells carry two missense mutations in the *TP53* (Fig. 1H). The c.524G > A mutation substitutes arginine with histidine at position 175 (exon 5), and the c.743G > A mutation substitutes arginine with glutamine at position 248 (exon 7). Both mutations are hotspot mutations and are located within the DBD of p53 [29]. Mutations in this domain can disrupt p53's ability to bind to specific DNA sequences and thereby affect its transcriptional activity [29]. CCRF-CEM cells were found to be highly resistant to RG7388 (Fig. 1F), consistent with the recently published study [30], indicating that these mutations severely compromise p53 function, likely through loss of DNA binding capacity or impaired interaction with other regulatory proteins critical for MDM2-mediated regulation. In summary, the sensitivity or resistance to RG7388 in these cell lines correlates with the functional consequences of the *TP53* mutations they harbor, particularly considering the location

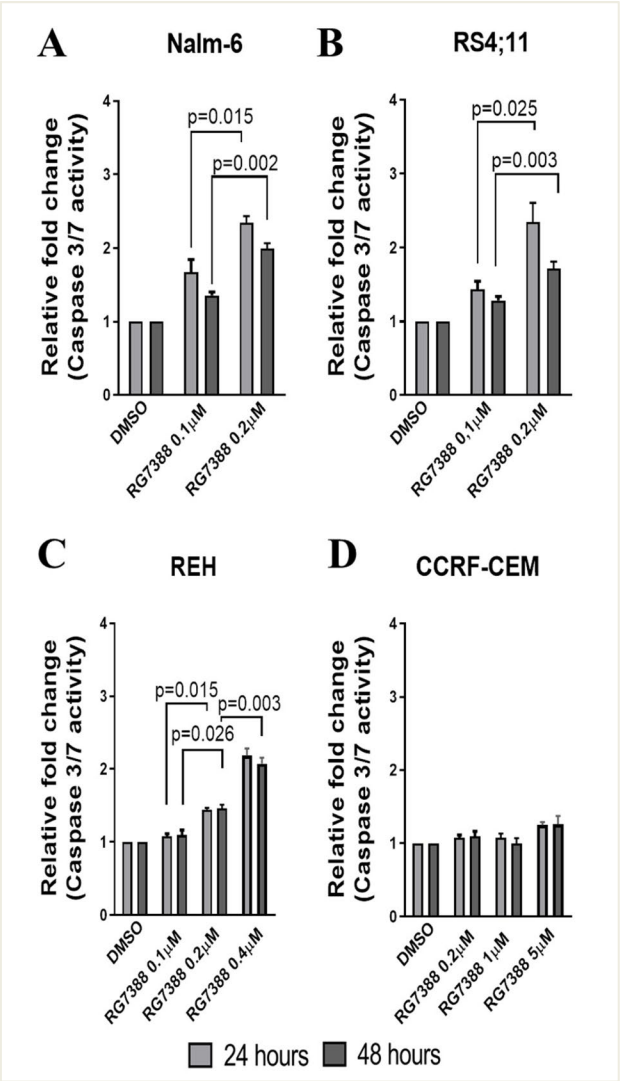

Fig. 3 Caspase 3/7 activity as an indicator of apoptosis. Caspase 3/7 activity is represented as fold change relative to DMSO solvent control. ALL cell lines **(A)** Nalm-6, **(B)** RS4;11, **(C)** REH and **(D)** CCRF-CEM were treated with the RG7388 concentrations indicated on the chart for 24 and 48 h. Data are shown as the average of at least 3 independent repeats and error bars represent SEM. Statistically significant *p*-values are indicated

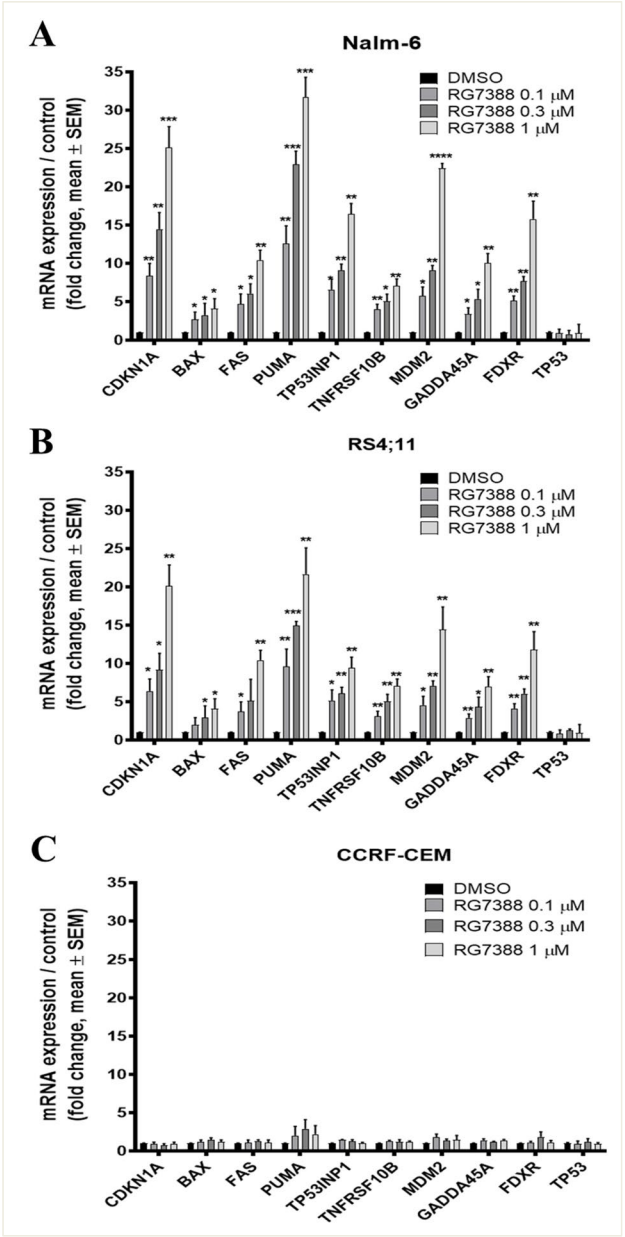

Fig. 4 The mRNA expression of p53 transcriptional target genes by qRT–PCR. The mRNA expression of p53 transcriptionally regulated genes in response to 0.1, 0.3 or 1 μM RG7388 for 6 h relative to DMSO solvent and GAPDH control in **(A)** Nalm-6, **(B)** RS4;11 and **(C)** CCRF-CEM cells. Statistically significant differences (*$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$) are shown above each bar for each treatment compared with DMSO control. Data are presented as mean ± SEM for three independent repeats

of these mutations within the DNA-binding domain of the p53 protein [31]. Truncating mutations like p.R306* may retain some residual sensitivity, whereas missense mutations like p.R175H and p.R248Q can lead to severe resistance due to impaired p53 function.

RG7388 disrupts the MDM2-p53 interaction, leading to p53 stabilization and activation in both *TP53* wild-type (Nalm-6 and RS4;11) and mutant (REH) ALL cells (Fig. 2A). This activation induces $p21^{WAF1}$ transcription (Fig. 2A), regulating cell cycle progression [32]. RG7388 also upregulated MDM2 levels in a concentration-dependent manner in *TP53* wild-type cells (Fig. 2B), due to an autoregulatory feedback loop [33]. The induction of PUMA and cleavage of PARP-1 support RG7388's ability to induce apoptotic cell death via p53-mediated mechanisms in *TP53* wild-type ALL cells. MOLT-4 cells did not exhibit functional p53 activation (Fig. 2A), indicating potential regulatory mechanisms or post-translational modifications may

affect p53 functionality differently compared to other drug-responsive cell lines. As an additional point of relevance, our earlier preclinical research focused on chronic lymphocytic leukemia identified the splicing factor gene *SF3B1* as a potential contributor to the RG7388 resistance, alongside the *TP53* status, which remains a key indicator of response to MDM2 inhibitors [34]. Interestingly, MOLT-4 cells harbor multiple missense mutations in the *SF3B1* gene, as indicated by the Cancer Sanger Database. This suggests the presence of splicing variants potentially affecting the p53 pathway in these cells, which may partially explain the absence of functional p53 evidence in Western blot analyses despite the cells' sensitivity to the drug. Further research in this area could be highly valuable.

The concentration-dependent activation of caspase 3/7 observed in idasanutlin-treated cells provides mechanistic insights into its apoptotic effects (Fig. 3). Activation of caspases, particularly caspase 3 and 7, is a hallmark of apoptotic cell death and is indicative of idasanutlin's ability to induce both intrinsic (mitochondrial-mediated) and extrinsic (death receptor-mediated) apoptotic pathways [25]. The consistent apoptotic response observed between the 24-hour and 48-hour treatments suggests that RG7388's apoptotic effect reaches a plateau within the initial 24 h of exposure, with no substantial increase over prolonged treatment periods. This stability in apoptotic activity illustrates the robustness of RG7388-induced apoptosis in sensitive ALL cell lines and suggests that shorter treatment durations may be sufficient to achieve maximal therapeutic benefit.

qRT-PCR analysis further elucidated RG7388's impact on p53 transcriptional activity by evaluating mRNA expression levels of cell cycle arrest and apoptotic genes. Consistent with Western blot findings, RG7388 treatment induced robust upregulation of *CDKN1A* (p21) and pro-apoptotic genes (*BAX*, *FAS*, *PUMA*, *TP53INP1*, *TNFB-SF10B*) in Nalm-6 and RS4;11 cells harboring wild-type *TP53* (Fig. 4A-B). These findings highlight RG7388's role in enhancing p53-mediated transcriptional responses critical for inhibiting cell proliferation and promoting apoptosis in ALL. Conversely, the lack of significant gene induction in CCRF-CEM cells further emphasizes the genotype-specific effects of RG7388 (Fig. 4C).

Our findings are consistent with previous studies demonstrating idasanutlin's efficacy in various cancer types with wild-type *TP53* [18–20, 35], highlighting its potential as a targeted therapy. The specificity of idasanutlin for *TP53* wild-type ALL cells suggests its potential clinical utility in settings where *TP53* mutations are infrequent, such as in pediatric ALL populations [7, 30]. This study contributes to the growing body of evidence supporting MDM2 inhibition as a strategy to reactivate p53 and induce apoptosis in cancer cells. This approach is particularly relevant in hematologic malignancies, where apoptosis evasion is a common mechanism of resistance to standard therapies [36–39]. Future clinical trials should focus on validating idasanutlin's efficacy and safety profiles in patient populations with confirmed *TP53* wild-type status. Biomarker studies are crucial to identify predictive markers of idasanutlin response, potentially enabling personalized treatment strategies. Integration of genomic and molecular profiling techniques could facilitate the identification of patients most likely to benefit from idasanutlin therapy. Moreover, combination therapies incorporating idasanutlin with standard chemotherapeutic agents or novel targeted therapies [40–42] warrant investigation to enhance treatment outcomes and mitigate resistance mechanisms. Preclinical models should be employed to explore synergistic effects and optimize dosing regimens that maximize therapeutic efficacy while minimizing toxicity.

While this study highlights RG7388's potential in targeting ALL cells with different *TP53* statuses, limitations include the absence of clinical validation. Nevertheless, the study's strengths are evident in its thorough evaluation of genotype-specific responses and its comprehensive approach, utilizing various assays to assess RG7388's efficacy and mechanism. These findings support RG7388's potential for targeted therapy and provide a foundation for future clinical research. The study's key strengths include its detailed analysis of RG7388's effects across different *TP53* mutations and its use of various assays. Although REH cells harbor a mutant p53, they remained sensitive to the growth arrest induced by RG7388. Additionally, caspase 3/7 enzymatic activity suggested that apoptosis was triggered in the REH cell line following RG7388 treatment. Therefore, examining the possible genes involved in apoptosis in this cell line could be a focus of future research, potentially utilizing qRT-PCR for further investigation.

Conclusions

In this study, we explored the therapeutic potential of idasanutlin in human ALL cell lines. Our findings demonstrate its ability to induce growth inhibition in ALL cells with both wild-type and mutated *TP53*. Idasanutlin treatment resulted in concentration-dependent activation of caspase 3/7, signaling apoptotic cell death through intrinsic and extrinsic pathways. These results highlight the need for further preclinical and clinical investigations to confirm idasanutlin's efficacy and safety across various hematologic malignancies. Our study contributes to the growing evidence supporting MDM2 inhibition as a strategy to restore p53 function and promote apoptosis in cancer cells. Future research should focus on identifying predictive biomarkers for idasanutlin response and optimizing combination therapies to improve outcomes in ALL and other *TP53* wild-type cancers. In

conclusion, idasanutlin shows promise as a novel therapeutic agent against *TP53* wild-type ALL, warranting continued exploration in clinical settings.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10637-024-01473-9.

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Author contributions E.A. was responsible for the experimental design and supervised the project. E.A. secured the project budget. S.G. and E.A. carried out the experiments. E.A. provided the material resources. S.G. and E.A. wrote the original draft and analysed the data. S.G. and E.A. have read and agreed to the final version of the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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