MDM2 antagonists as a novel treatment option for acute myeloid leukemia: perspectives on the therapeutic potential of idasanutlin (RG7388)

Abstract: Acute myeloid leukemia (AML) is a clonal heterogeneous malignancy of the myeloid cells with a poor prognosis lending itself to novel treatment strategies. TP53 is a critical tumor suppressor and plays an essential role in leukemogenesis. Although TP53 is relatively unusual in de novo AML, inactivation of wild-type p53 (WT-p53) is a common event. Murine double minute 2 (MDM2) is a key negative regulator of p53 and its expression; inhibition of MDM2 is postulated to reactivate WT-p53 and its tumor suppressor functions. Nutlins were the first small molecule inhibitors that bind to MDM2 and target its interaction with p53. RG7388 (idasanutlin), a second-generation nutlin, was developed to improve upon the potency and toxicity profile of earlier nutlins. Preliminary data from early phase trials and ongoing studies suggest clinical response with RG7388 (idasanutlin) both in monotherapy and combination strategies in AML. We herein briefly discuss currently approved therapies in AML and review the clinical data for RG7388 (idasanutlin) and MDM2 inhibition as novel treatment strategies in AML. We further describe efficacy and toxicity profile data from completed and ongoing trials of RG7388 (idasanutlin) and other MDM2-p53 inhibitors in development. Many targeted therapies have been approved recently in AML, with a focus on the older and unfit population for intensive induction therapy and in relapsed/refractory disease. The “nutlins”, including RG7388 (idasanutlin), merit continued investigation in such settings.

Keywords: AML, myeloid leukemia, nutlins, MDM2, idasanutlin, RG7388, p53 inhibitor

Introduction
Acute myeloid leukemia (AML) is the most common acute leukemia in adults. It is much more prevalent in the elderly with a median age at diagnosis of 68 years. Surveillance, Epidemiology and End Results Program (SEER) 2018 analysis estimates approximately 19,520 new cases of AML (1.1% of all cancers) and 10,670 deaths (1.8% of all cancers) in the US. AML is a clonal disorder with heterogeneous molecular, cytogenetic, biological and clinical features. Significant progress has been made in the last 4 decades understanding the genomic landscape of AML. This has translated into remarkable growth in drug development and approval. After a very long drought in AML therapeutics, we have witnessed recent FDA approval for multiple new drugs. These new agents target molecular drivers of AML such as Fms-like tyrosine kinase 3 (FLT3), epigenetic regulators such as isocitrate dehydrogenase (IDH1/2) and monoclonal antibodies against surface markers on leukemia cells such as CD33. Many other similar agents are currently in clinical trials. One such target in AML is exploring the inhibition of the interaction between tumor suppressor gene p53 and murine double minute 2 (MDM2). We herein briefly review...
the current AML treatment paradigm and explore in detail the pharmacology, mechanism of action and efficacy of the MDM2 inhibitor RG7388 (idasanutlin).

Despite recent advances, the 5-year survival rate for AML remains at 25%–30%. Standard of care for younger and fit patients eligible to undergo aggressive induction therapy remains the “7+3” regimen, 7-day continuous cytarabine infusion and 3 daily doses of an anthracycline agent, followed by consolidative strategies with high-dose cytarabine or allogeneic stem cell transplantation (Allo-SCT). Older and unfit patients not suitable for aggressive treatments have dismal outcomes with median overall survival (OS) less than 1 year.16,17 There are limited options for older and unfit patients including best supportive care or hypomethylating agents. The FDA has recently granted accelerated approval to venetoclax in combination with azacitidine, decitabine or low-dose cytarabine in newly diagnosed AML patients older than 75 years or with comorbidities precluding the use of intensive induction chemotherapy.18 Although this novel approach is potentially paradigm shifting, the majority of patients will relapse. There is a great need for more effective therapies.

For patients with relapsed/refractory (R/R) disease, there are often fewer options. Given the inadequacy of current treatments, enrollment in clinical trials is always recommended whenever possible. Selected patients are able to undergo more intensive chemotherapy with a goal of transplant if eligible. However, for many patients, cytotoxic chemotherapy is not appropriate or has a low likelihood of success. In a Phase III trial evaluating elacytarabine, novel cytarabine, versus the investigator’s choice of 1 of the 7 commonly used AML salvage regimens, the median survival was approximately 3 months in both arms.19 That sobering statistic is an impetus for new drug development. Many of the recent FDA approvals in AML are targeted agents in the R/R setting. FLT3 is a tyrosine kinase receptor involved in normal hematopoiesis and cell proliferation. Activating FLT3 mutations have been reported in up to 30% of the AML patients including 20% with internal tandem duplication (FLT3-ITD) mutations and 5%–10% with point mutations in the activating loop of the tyrosine kinase domain (FLT3-TKD). Gilteritinib, an oral FLT3 inhibitor, was recently approved for adults with FLT3-positive R/R AML.20 In the ADMIRAL study that led to FDA approval, the rate of complete remission (CR) or CR with partial hematologic recovery (CRh) was 21%. Other highly selective FLT3 inhibitors such as quizartinib and crenolanib are in clinical trials both in the upfront and R/R settings. Disordered epigenetic regulation also has therapeutic potential. Both IDH1 (ivosidenib) and IDH2 (enasidenib) inhibitors are now approved in the R/R setting, in which they have clearly shown a survival benefit and improvement in the quality of life with transfusion independence and less febrile neutropenia requiring hospitalizations. Response rates for both agents approximate 30%–40%. Multiple other targets are under investigation at this time including p53-MDM2 inhibitors-nutlinis.

p53 and MDM2

TP53 mutations occur in about 7%–8% of the de novo AML cases, whereas inactivation of wild-type p53 (WT-p53) occurs in almost all AML subsets.2 p53 transcription factor plays a crucial role in tumor suppression by various mechanisms including apoptosis, DNA repair, maintenance of normal stem cell pool and regulating self-renewal, thereby preventing leukemogenesis in AML.21–24 TP53, the gene encoding p53, is known to be mutated in up to 50% of all human cancers.25 Kemp et al26 demonstrated that a lack of p53 led to the increased predisposition of various tumors in murine models. Multiple mechanisms have been described for inactivation of WT-p53. The best studied of those is through MDM2 overexpression and p14 (ARF) inactivation. The ARF-MDM2/p53 axis is involved in most AML cases, with ARF inactivation or MDM2 overexpression leading to non-functional p53.27

MDM2 serves as a negative regulator of p53. Wu et al28 first described the mutual regulation between p53 and MDM2 through a feedback loop. Activation of p53 through any stimuli or DNA damage increases transcription of MDM2 mRNA and protein which in turn binds to p53 and directly inhibits its function via 3 primary mechanisms. First, the E3 ligase activity of MDM2 directly ubiquitinates p53 leading to its degradation through proteasomes. Second, MDM2 and p53 binding blocks p53 from binding to its target DNA causing lack of transcription. Third, MDM2 increases the export of p53 from the cell nucleus that makes it inaccessible to the target DNA for transcription.29,30 In vivo studies have also confirmed this interaction and the oncogenic potential of MDM2 overexpression, with increased expression leading to increased tumor formation.31–33 Oliner et al34 first described MDM2 amplification in one-third of the human sarcoma samples. Momand et al35 showed similar amplification in multiple other tumor types. Besides gene amplification, another mechanism for MDM2 overexpression was described by Bond et al36,37 as single nucleotide polymorphism (SNP) in the MDM2 promoter region. MDM2 gene amplification

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal is indexed on PubMed Central and Embase.

Submit your manuscript | www.dovepress.com
Dovepress

OncoTargets and Therapy downloaded from https://www.dovepress.com/ by 54.70.40.11 on 11-Jun-2020
For personal use only.
remains the most essential implicated mechanism in MDM2 overexpression.\textsuperscript{40} More importantly, MDM2 gene amplification and TP53 mutations are mutually exclusive in human cancers.\textsuperscript{36,41,42} Of note, preclinical data suggest that about two-thirds of AML cell lines and patient-derived samples are sensitive to MDM2 inhibition, and as expected, the TP53 mutated cells show resistance.\textsuperscript{43,44}

Based on the MDM2-p53 interaction, inhibition of MDM2 was postulated to reactivate WT-p53 and its tumor suppressor functions, making it a potential therapeutic target. Momand et al\textsuperscript{45} mapped the MDM2-p53 protein–protein interaction to the first 120 amino-terminal amino acid residues of MDM2 and the first 30 amino-terminal residues of p53. In 2004, Vassilev et al\textsuperscript{46} first discovered “nutlins”, the small molecule inhibitors that bind to MDM2 and target its interaction with p53. In vivo studies of nutlin-3 showed extensive reduction in tumor mass in the MDM2-amplified xenograft osteosarcoma model.\textsuperscript{47} Pishas et al showed significant apoptotic responses on immunohistochemical analysis of nutlin-3 treated human sarcoma tissue samples.\textsuperscript{48} These preclinical data led to the development of several potent and selective non-peptide small-molecule MDM2 inhibitors. The first MDM2 inhibitor to be advanced into human clinical trials was RG7112 (Hoffmann La Roche RO5045337).\textsuperscript{49} RG7112 is several times more potent and selective for WT-p53 than nutlin-3; furthermore, it demonstrated efficacy in both in vitro and in vivo studies and had a dose-dependent effect on tumor regression. In several Phase I trials with both solid and hematological malignancies, RG7112 showed evidence of on-target activity resulting in p53 activation. After treatment with RG7112, there was an increased expression of downstream pro-apoptotic proteins.\textsuperscript{50–52} In AML, RG7112 was studied both as monotherapy and in combination with low-dose cytarabine.\textsuperscript{53} Some patients even achieved CR and were subsequently transplanted. Dose-limiting toxicities (DLTs) noted in the combination trials were rash, thrombocytopenia, and diarrhea (>20% of the adverse events [AEs] were gastrointestinal [GI] or infectious). The hematological toxicity with this drug was prolonged, as MDM2 plays a crucial role in hematopoiesis.\textsuperscript{54} The higher dose to attain satisfactory p53 activation caused significant toxicities (cytopenias, diarrhea, sepsis, and deaths), and so the need for a more potent and less toxic agent was identified.

**RG7388 (idasanutlin)**

RG7388 (idasanutlin) is a second-generation MDM2 inhibitor. It was developed to improve upon the stereochemical and conformational properties of the spiroindole series by the introduction of the cyanopyrrolidine core, which was thought to be more flexible.\textsuperscript{55,56} It was found to be more potent, more selective, and had a better pharmacokinetic (PK) profile as compared to RG7112.\textsuperscript{57} It also showed dose-dependent p53 stabilization, apoptosis, and cell cycle arrest. In SJSa1 osteosarcoma xenografts in nude mice, RG7388 (idasanutlin) was more effective than RG7112 at much lower doses.\textsuperscript{56,57} RG7388 (idasanutlin) has also been studied in both solid and hematological malignancies. Here, we limit our discussion for its use and implications in AML.

In a multicenter Phase 1/1b study, RG7388 (idasanutlin) was evaluated in AML patients as monotherapy (daily for 5 days every 28 days) and in combination with cytarabine (ara-C 1 gm/m\textsuperscript{2} IV ×5 days every 28 days) in a dose escalation study.\textsuperscript{58} An extension cohort was initiated in both groups at the recommended Phase II dose (RP2D). The monotherapy extension arm included patients older than 70 years and patients older than 60 years with comorbidities. The combination extension arm included R/R patients with not more than 2 prior regimens. Patients with antecedent hematologic disorders or transplant were not eligible for the combination arm. The RP2D for RG7388 (idasanutlin) as monotherapy or in combination was determined to be 1,200 mg/day (600 mg bid for 5 days every 28 days). Only 1 DLT of prolonged myelosuppression was reported. The most common AEs were diarrhea and infection. Diarrhea was reported by greater than 85% of the patients and did not appear to be dose-dependent.

In the monotherapy arm, 9 patients were treated at the RP2D. The median age was 75 years (range 66–83); 8 of the 9 patients were reported to have an antecedent hematologic disorder. There were 3 responses including 1 complete response with incomplete recovery (CRi), 1 partial response (PR) and 1 hematological improvement (HI). There were 3 patient deaths in the first 30 days. Enrollment onto the monotherapy expansion phase was discontinued for prolonged myelosuppression which increased the risk of infection and early death. In the reported PK data, the t\textsubscript{1/2} was noted to be ~1 day and was irrespective of age, concomitant cytarabine, or azoles.\textsuperscript{58}

Seventy-six patients were treated on the combination arm in the dose escalation (n=23) and dose expansion cohorts (n=21) with an additional 32 patients in a bridging cohort.\textsuperscript{59} The bridging arm was added to characterize the safety and PK profile of a spray-dried powder formulation of RG7388 (idasanutlin). The CR rate was 25% (n=19); the composite CR rate (cCR, CR + CRp + CRi) was 29%. The cCR patients were followed until relapse or 1 year from

**Dovepress**

Journal of Medical Screening | 2019:12

submit your manuscript | www.dovepress.com

2905

OncoTargets and Therapy downloaded from https://www.dovepress.com/ by 54.70.40.11 on 11-Jan-2020

For personal use only.
the start of therapy; the median duration of response was ~6.4 months (1.1–11.9 months). Five patients remained in CR at 1 year follow-up. Patients with cCR also had minimal residual disease (MRD) assessment with multiparametric flow cytometry on day 28 and for those experiencing cCR at subsequent assessments. Median progression-free survival (PFS) for cCR patients was 315 days compared to 43.5 days in the non-responders. When MRD thresholds of <1% and >1% were applied, there was a statistically significant association with median PFS. Patients with MRD <1% had a median PFS of 367 days versus 84 days (p-value=0.001) in the MRD >1% group. The MRD findings provide further support of its utility in providing prognostic information in AML. To identify possible biomarkers of response, MDM2 protein expression was evaluated by intracellular flow cytometry on peripheral blood leukemic blasts and stem cells. Higher MDM2 expression in both leukemic blasts and stem cells was associated with CR; TP53 mutational status alone was not. These results raise the potential of MDM2 expression in leukemic cells to serve as a predictive biomarker for response. Interestingly, responses were identified in patients with TP53 mutations including 1 CR, suggesting TP53 mutation as an inadequate companion diagnostic for AML patients. Zhong et al previously reported similar results in in vitro AML cell lines but were based on whole blood samples instead of expression on leukemic cells only. Given the efficacy in Phase I/ib AML study, RG7388 (idasanutlin) is currently undergoing evaluation in a Phase III trial in combination with cytarabine versus cytarabine alone for R/R AML patients (NCT02545283).

RG7388 (idasanutlin) is also being extensively explored in combination with other apoptotic agents such as the BCL2 inhibitor venetoclax. In preclinical studies in p53 wild-type AML tumor models, the combination of RG7388 (idasanutlin) and venetoclax was synergistic. Similar results were seen in WT-p53 AML cell lines treated with the MDM2 inhibitor SAR405838 and bcl-2 inhibitor ABT-263 (navitoclax). Cell viability and annexin binding assays showed not only synergism but also potentiating efficacy. Interestingly, RG7388 (idasanutlin) induced G1 arrest and caused nuclear fragmentation in the G1 phase of the second cycle while the bcl-2 inhibitor caused apoptosis in G1 compartments. Cells that were transiently missed from apoptosis by RG7388 (idasanutlin) were hit by the bcl-2 inhibitor. Further studies with the combination suggest that each agent can reciprocally overcome the apoptotic resistance to either agent alone. The RG7388 (idasanutlin) and venetoclax combination is being evaluated in Phase I/ib trial for patients 60 years and older with R/R AML who are not candidates for cytotoxic therapy (NCT02670044). A recent Phase I study of the pegylated intravenous prodrug of idasanutlin (RO6839921) suggested a similar PK profile to RG7388 (idasanutlin). Antileukemic activity was noted to be around 42% in the overall population (26 patients).

Toxicity profile
Overall, RG7388 (idasanutlin) appears to be well tolerated. The most common AEs in the reported studies were limited to diarrhea, nausea and vomiting and myelosuppression causing febrile neutropenia and thrombocytopenia. These are thought to be the result of on-target effects of the drug on the normal cells. This toxicity profile is similar among all the MDM2 inhibitors. Long-term and off-target toxicities of these agents are currently unknown and will become evident with time.

Other MDM2 inhibitors
Other small molecule MDM2 inhibitors are currently undergoing investigation in AML. MK-8242 (SCH-900242) is an orally bioavailable and potent small molecule inhibitor of the MDM2-p53 interaction. In a Phase I study with 24 evaluable patients in R/R AML, MK-8242 was evaluated in 2 different schedules. Two DLTs were identified, bone marrow failure and prolonged cytopenias; no MTD was identified. The most common AEs of any grade were G1 and hematologic which was similar to other MDM2 inhibitors. Efficacy was modest with 1 PR, 1 CRi and 1 MLFS. AMG232, a potent oral MDM2 inhibitor, has also been evaluated in adults with R/R AML. In a Phase Ib study, AMG232 with or without trametinib (MEK inhibitor) was administered to 35 patients. There was some early evidence of activity but no reported CRs in the monotherapy arm; there was, however, 1 CR in the combination arm. An MTD was identified; toxicities were again similar to those seen with other MDM2 inhibitors. AMG-232 is currently being evaluated in combination with decitabine in newly diagnosed AML patients with WT-p53. DS-3032b (milademeten) is another oral p53-MDM2 inhibitor currently under evaluation in patients with AML and other hematological malignancies. In a Phase I dose escalation study in 38 patients with R/R AML and high-risk myelodysplastic syndrome (MDS), the single-agent MTD was determined to be 160 mg daily 21/28 days. The toxicity profile was similar to other MDM2 inhibitors with GI and hematological toxicity; 5 subjects had DLTs including grade 3 hypokalemia, grade 3 diarrhea, grade 3 nausea and vomiting, grade 2 renal insufficiency...
and grade 3 anorexia/fatigue. There were CRs in 2 patients with AML and 1 patient with MDS achieved a marrow CR. It is notable that each patient developed a TP53 mutation while on treatment. Further evaluation with azacitidine is ongoing (NCT02319369). DS-3032b is also being evaluated in combination with cytarabine and quizartinib in AML patients (NCT03552029).

More recently, dual inhibition of MDM2/MDMX as a therapeutic strategy is undergoing clinical evaluation. MDMX similar to MDM2 also represses TP53 transcriptional activity. It is hypothesized that targeting TP53 interactions with MDM2 and MDMx will have a more significant impact than MDM2 inhibition alone. ALRN-6924 is a novel “stapled peptide”, which has been structurally stabilized in an α-helical configuration, to mimic the inhibitor binding region of TP53. By mimicking this region, ALRN-6924 can bind the 2 most important endogenous inhibitors of p53, MDM2 and MDMX. In Phase I/ib study, ALRN-6924 was evaluated alone and in combination with cytarabine in AML patients. In the preliminary results for 13 monotherapy and 19 combination arm patients, there were no DLTs and MTD was not identified. The most common AEs were notably GI toxicity and thrombocytopenia. In the 27 efficacy evaluable patients, there were 2 marrow CRs in 4 MDS patients; 1 AML patient had a 50% reduction in marrow blasts. Ongoing studies of MDM2 small molecule inhibitors and MDM2/MDMX stapled peptide drugs are listed in Table 1.

**Challenges and future directions**

MDM2 inhibition is a promising therapeutic target in AML. Long-term data are needed to further elucidate the potential toxicities, mechanisms of resistance and efficacy. Challenges that have been identified are related to its on-target effects to normal cells especially GI and hematological toxicities and the emergence of resistance. Data from the clinical studies to date suggest late hematological toxicity due to on-target effects on the bone marrow. Identifying the optimal dose for each MDM2 inhibitor during monotherapy as well as in combination especially with agents such as venetoclax which is known to cause myelosuppression is necessary.

Development of resistance to MDM2 inhibitors seems to occur due to the emergence of p53 mutations through a selection of p53-mutated clones or the emergence of p53 mutation. Other mechanisms described are through point mutations in the p53-binding pockets of MDM2 and high MDMX (positive regulator of MDM2) levels. In vitro, the AML cell lines that are treated with MDM2 inhibitors and develop resistance still retain sensitivity to BCL-2 inhibitors. Hopefully, the combination with bcl-2 inhibitors will overcome this issue, although clinically such resistance is yet to be described. This again demonstrates the efficacy of combination strategy over monotherapy by targeting different apoptosis mechanisms simultaneously or sequentially.

The clinical relevance and applicability of targeting apoptotic mechanisms in AML have been confirmed with the recent approval of venetoclax (bcl-2 inhibitor) in combination with azacitidine, decitabine or low-dose cytarabine for newly diagnosed AML patients aged 75 or older who are not candidates for intensive induction. In Phase I/II studies, the combination showed overall response rates of 60%–70%. Data are awaited on most of the combination studies for idasanutlin as the trials are ongoing. The challenge remains how best to incorporate it with currently approved therapies and which population to target for maximal benefits. To date, it seems to be well tolerated as monotherapy in older individuals not candidates for intensive chemotherapy, but efficacy results have been modest. It could potentially be incorporated in combination with induction therapy for younger/fit adults to improve response.
especially in those with poor risk or chemotherapy refractory disease.\textsuperscript{67}

Precision medicine is the future of oncology. AML patients are beginning to benefit from this approach with the recent approval of FLT3, IDH1/2 inhibitors. However, not all patients harbor a targetable mutation; for those patients, targeting the apoptosis pathway may prove to be an effective alternative. Further studies are required to further understand the mechanisms of resistance, toxicity and biomarkers for the prediction of response and prognosis.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**


62. Khurana and Shafer
75. Jones RJ, Bjorklund CC, Baladandayuthapani V, Kuhn DJ, Orlowski RZ. Drug resistance to inhibitors of the human double minute-2 E3 ligase is mediated by point mutations of p53, but can be overcome with the p53 targeting agent RITA. *Mol Cancer Ther.* 2012;11:2243–2253. doi:10.1158/1535-7163.MCT-11-0824-T

