Acute Myeloid Leukemia Patients' Clinical Response to Idasanutlin (RG7388) Is Associated with Pre-Treatment MDM2 Protein Expression in Leukemic Blasts

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In translational research described, we investigated biomarker expression by flow cytometry for MDM2 antagonist clinical response association in relapsed/refractory AML patients treated with idasanutlin-based therapy (Clinicaltrials.gov identifier: NCT01773408). As MDM2 targets p53 for ubiquination/degradation, higher levels of MDM2 protein expression would be consistent with a mechanism of increased malignant potential by decreased p53 activity. We hypothesized that this comprises a mechanism for oncogene addiction whereby the cell is uniquely sensitive to enhanced p53 activity by MDM2 antagonism through reliance on this pathway. Flow cytometry assessment of MDM2 protein expression in leukemic blasts (CD45dim or CD45dim/CD34+) is established in both a proof of principle set comprising initial trial segments, and then validated in a trial segment comprising patients treated with a clinically optimized idasanutlin formulation. Upon TP53 WT patient subset analyses, we show that flow cytometry assessments add predictive/prognostic value to negative predictive value conferred by TP53 mutational status. Additionally, we propose that such continuous variable measurements are consistent with FDA guidance for the development of complementary diagnostics. These flow cytometry assessments will be monitored in upcoming AML clinical trials for predictive diagnostic potential.

Despite considerable research efforts to identify novel therapies, outcomes in acute myeloid leukemia (AML) remain poor due to limited treatment options. MDM2 antagonism represents a unique and promising approach for the treatment of solid tumors and acute leukemias. The earliest MDM2 antagonists, termed nutlins, disrupted MDM2 targeting of p53 for ubiquination and degradation, thus stabilizing the p53 protein to exert tumor suppression transcriptional regulation and cause induction of apoptotic pathways. The identification of AML cases in which functional p53 activation drives efficacy may translate into improved patient outcomes for treatment with idasanutlin. Cells that have acquired a selective growth advantage through diminished p53 functional activity by amplification or overexpression of MDM2 may offer a unique paradigm for targeting a co-opted mechanism by oncology therapeutics.

Previously, MDM2 antagonism efficacy modeling was analyzed using large oncology cell line panels, with resulting multi-marker algorithms associated with response. In vitro MDM2 antagonist activity associated with a 4-gene panel (MDM2, XPC, CDKN2A /p16, BBC3/PUMA), each regulated by p53, was validated in AML clinical studies with previous (RG7112) and current (idasanutlin [RG7388]) MDM2 antagonists in clinical development. Interestingly, in an analysis of blood specimens from patients with AML, none of the 4 genes alone showed significant association with patient outcomes following treatment with MDM2 antagonists. When a multigene algorithm was considered, however, an association with response was identified. Because these results were from the whole blood of AML patients, signal association of individual genes may be obscured by non-AML contributions within the matrix. Thus, we examined MDM2 at the protein level using multiparametric flow cytometry refined to AML leukemic blasts.

Idasanutlin (RG7388) is a potent and selective MDM2 antagonist showing promising responses in phase 1 studies in relapsed/refractory AML. Trial NP28679 (NCT01773408) is a phase 1/1b study evaluating idasanutlin treatment (monotherapy or in combination with cytarabine) in relapsed or refractory AML patients. Safety and pharmacokinetics were primary endpoints, with clinical response as a secondary endpoint. Idasanutlin was generally well tolerated with GI toxicity being the most commonly reported adverse event (manageable by prophylaxis treatment.) Patient responses were observed with monotherapy and combination with cytarabine with some CR durations > 12 months. We tested pretreatment peripheral blood specimens from idasanutlin-treated patients for both TP53 mutations and MDM2 percent cell positivity using intracellular flow cytometry (described in the Online Supplementary Appendix) gated on CD45dim blasts and CD45dim/CD34+ leukemic blast subpopulations (depictions of gating in Online Supplementary Figures S1 and S2). These results were then correlated with idasanutlin-based therapy clinical outcomes.

A proof of principle training data set comprised patients treated with idasanutlin monotherapy (400-1600 mg/day for 5 consecutive days within a 28-day cycle; microprecipitated bulk powder formulation) or in combination with cytarabine 1 g/m2 IV on days 1-6 during the trial segment comprising patients treated with idasanutlin (RG7388) is associated with pre-treatment MDM2 protein expression in leukemic blasts.
first part of study NP28679. Eighty-nine patients with AML were treated with idasanutlin-based therapy, with 75 evaluable responses. Of these patients, flow cytometry data were available for 63 patients. We observed that MDM2 expression in leukemic blasts was significantly associated with patients exhibiting a composite complete remission (CRC; consists of CR, CR with incomplete platelet recovery [CRp], and CR with incomplete hematologic recovery [CRi]) vs. no response (progressive disease, hematologic improvement, and partial response) (Figure 1 and Table 1A; n=63; Wilcoxon P=0.0039; area under the curve [AUC], 0.73; 95% CI, 0.57-0.89). MDM2 percent cell positivity in CD45dim/CD34+ cells also showed association with clinical outcomes (n=62; Wilcoxon P=0.024 (AUC, 0.68; 95% CI, 0.49-0.87).

For validation of the proof of principle observation, a subsequent group of patients treated with an optimized idasanutlin formulation was assessed. In this cohort, 32 patients were treated with 300 mg twice daily or 400 mg twice daily on days 1-5 in combination with cytarabine,
with response assessment at the completion of cycle 1 (after day 28). Twenty-nine of 31 response-evaluable patients had flow cytometry data. Association of CRc with MDM2 expression in CD45dim blasts was also shown in this separate patient group (Figure 1 and Table 1A; Wilcoxon \( P = 0.0062 \); AUC, 0.78; 95% CI, 0.60-0.95). Comparable results validated the initial observations from the proof of principle set analysis of response association with MDM2 protein expression in the CD45dim/CD34+ blast subpopulation of AML patients (Wilcoxon \( P = 0.0016 \); AUC, 0.82; 95% CI, 0.65-0.99).

In total, for all AML patients from trial NP28679 with assessable clinical response and flow cytometry assessments (n=91), the association of percent cell MDM2 positivity with clinical response in both total CD45dim cells (Figure 2 and Table 1B; Wilcoxon \( P = 0.00019 \); AUC, 0.74; 95% CI, 0.62-0.85) and CD45dim/CD34+ cells was significant (Wilcoxon \( P = 0.00046 \); AUC, 0.73; 95% CI, 0.61-0.84).

The Online Supplementary Table S1 lists patient clinico-pathologic information. Of note, TP53 mutation (defined by next-generation sequencing (NGS) status) was associated with response (\( P = 0.018 \)). This association is derived from the negative predictive/prognostic impact of a small proportion of TP53-mutant patients (18 mutants/92 informative = 19.6%), of which only 1 had a CR, but is uninformative for the wild-type (WT) patients. When analyses of MDM2 percent cell positivity was restricted to only WT patients, significance was marked in both CD45dim (n=73; \( P = 0.00053 \); AUC, 0.73; 95% CI, 0.61-0.86) and CD45dim/CD34+ (n=72; \( P = 0.0016 \); AUC, 0.71; 95% CI, 0.59-0.84) cell subpopulations (Figure 2 and Table 1B). These results are consistent with the MDM2 protein acting as a biomarker for response distinct from TP53 mutation status that may contribute to predictive/prognostic outcomes discrimination.

Interestingly, the level of significance for clinical response association with MDM2 measurements taken from CD45dim blasts and the CD45dim/CD34+ subpopulation was similar. In additional flow cytometry analyses, we found that clinical response association with CD45dim/CD34+CD117+ leukemic blasts was significant for all patients (\( P = 0.00018 \)) as well as the TP53 WT subpopulation (\( P = 0.00054 \)). No significance was noted for differences between blast subpopulations analyzed and the level of significance for MDM2 expression/response association (pairwise comparisons, all \( P > 0.1 \)). These results are consistent with MDM2 protein expression measurements in leukemic blasts as holding association with outcomes, with insignificant differences among blast cell subpopulations.

The results of such continuous variable measurements stand in contrast to the binary nature of WT/mutant status that traditionally allow for straightforward interpreta-
tion in deployment of companion diagnostics in oncology patient management for many therapies. However, TP53 mutation is inadequate as an MDM2 antagonist companion diagnostic in AML because it conveys only negative predictive value in a small patient proportion (10-20%), while largely uninformative with regard to identifying responders among WT individuals. Additionally, some TP53 mutants display enhanced p53 activity following MDM2 antagonist treatment, leading to response. We propose that AML blast MDM2 expression may provide added guidance.

The development of such patient enrichment strategies aligns with the recent US Food and Drug Administration focus toward patient enrichment within the context of “complementary diagnostics,” rather than focusing on platform-centric binary guidance for response discrimination. It is proposed these measurements among AML cells may provide added guidance by employing a continuum of measurements against the likelihood of response, thus retaining the continuous variable component. Further assessment of the scope of the outcomes associated herein to extend to survival outcomes will be addressed in future AML trials. Strategies will also look beyond AML patient blood and extend to bone marrow aspirate for determining stromal cell interaction impact on biomarkers derived from targeted cell subpopulations.

In summary, the results presented herein support improved MDM2 antagonist clinical outcomes in AML patients with higher levels of MDM2 protein expression to modulate p53 activity. Such oncogene addiction derives from the reliance of a tumor on a dominant oncogene or mechanism for growth and survival, so that inhibition of this specific oncogene/pathway is sufficient to halt the neoplastic phenotype. Consistent with the concept of oncogene addiction, MDM2 protein expression from blasts may provide added guidance in identifying AML patients likely to exhibit improved outcomes to idasanutlin-based therapy. This biomarker will continue to be evaluated for diagnostic potential in future randomized clinical studies of idasanutlin.

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References