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MDM2 antagonist clinical response association with a gene expression signature in acute myeloid leukaemia

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Acute myeloid leukaemia (AML) is uniquely sensitive to p53 activation [1, 2] as ≈90% of patients carry wild-type *TP53* and frequent MDM2 overexpression.[3] MDM2 blocks p53 transactivation and targets p53 for ubiquitin-dependent degradation.[4, 5] Nutlins have been characterized as potent and selective small-molecule MDM2 antagonists.[1, 6–8] RG7112 was the first such MDM2 antagonist to undergo clinical assessment in solid tumors and leukaemia trials.[1, 2, 9] As not all patients with functional p53 will respond to MDM2 antagonists, diagnostic tools may identify patients likely to respond.

To establish an *in vitro* MDM2 antagonist therapy-predictive mRNA signature, we assessed genome-wide associations between growth inhibitory effects of RG7112 (IC_{50}s) among 287 human cancer cell lines and pretreatment RNAseq-derived transcript levels (Table S1, Figure S1). Thirty-five candidate genes were identified with significance at false discovery rate 0.05 through two approaches: comparing mRNA expressions between sensitive (IC_{50}<1μM) and resistant cell lines (IC_{50}>10μM); or Spearman correlation between mRNA expressions and IC_{50}s (Table S2). Functional annotation indicated that many significant genes were known regulators of the MDM2-p53 interaction or downstream p53 pathways, including cell-cycle arrest and apoptosis. Among these, *MDM2* demonstrated association between overexpression and *in vitro* sensitivity to RG7112 (Spearman correlation coefficient=−0.39; P<0.001). A multiple logistic regression classifier was identified comprising high expression of *MDM2, XPC, BBC3* (PUMA), and low expression of tumor suppressor gene *CDKN2A* (Table S3). This signature score ($G_{MDM2} + G_{XPC} + G_{BBC3} - G_{CDKN2A}$ at baseline), associated with cell-line response to MDM2 antagonist (P<0.001) and discriminated sensitive from resistant cell lines (area under the curve [AUC] = 0.92; 95% CI, 0.87–0.97; Table 2, S3 and Figure S2). In addition to *MDM2*, the other 3 signature components are

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regulators of the MDM2–p53 interaction or downstream p53 pathways. *XPC* is key in repairing damaged DNA. *BBC3* (PUMA) is induced by exposure to DNA-damaging agents and by activated p53, and mediates apoptosis. *CDKN2A* gene, comprising p16 and p14ARF, is linked to tumor suppressor pathways, inhibiting MDM2 function by nucleolus sequestering.[10] Cell lines with low signature scores trended with p53 mutation, whereas cell lines with high signature score trended with p53 wild type ($P<0.001$; Figure S2). Multivariate logistic models indicated signature scores remained significant ($P<0.001$) when adjusted for *TP53* mutation status.

RG7112 was assessed in phase 1 dose escalation trial NO21279 (patients with relapsed/refractory leukaemia; Figure S1;Table 1). Enrollment criteria are detailed in Supplement. Clinical response in NO21279 was as follows: responders were patients whose bone marrow blasts were <= 5% after treatment; >5% blasts were non-responders. mRNA expressions in blood leukaemia samples and bone marrow aspirate samples were profiled from 28 evaluable patients treated at the maximum tolerated dose (1500 mg/m^2 twice daily × 10 days) at pretreatment, after a single dose (cycle 1 day 2 [C1D2], blood only), and on last day of dosing (cycle 1 day 10 [C1D10]). Signature scores from pretreatment blood samples associated with clinical response ($P=0.005$; Table 1; Figure S2) and with pharmacodynamic biomarker response, defined as change in *MDM2* mRNA expression in blood (Spearman correlation coefficient 0.41; $P=0.02$; Figure S3). Signature scores distinguished response with AUC=0.86 (95% CI, 0.71–1.00); higher than AUCs of *TP53* mutation status or *MDM2* mRNA expression in blood as individual biomarkers (Table 2). Using a signature score cutpoint selected by Youden index, patients were classified by response prior to MDM2-antagonist therapy with 100% sensitivity and 65% specificity. ***TP53*-mutant patients showed a trend of lower signature scores than *TP53*–wild-type patients, although not significant ($P=0.068$; Table S4)**. Furthermore, signature scores of *TP53*-wild-type responders are significantly higher than *TP53*-wild-type non-responders ($P=0.006$; Table S4), demonstrating additional discriminative power of the proposed signature in *TP53*-wild-type patients. Correlation (P=0.02) was observed between clinical response and signature score in multiple logistic regression with both *TP53* mutation status and signature. Taken together, these data indicate the signature score can potentially serve as an indicator of MDM2–p53 pathway function, with added predictive value beyond TP53 status for AML patients.

We also sought to determine if the 4-gene signature may provide pharmacodynamic metrics for assessing clinical activity consistent with the intended mechanism of action Relative median expression of *MDM2, XPC, BBC3*, and *CDKN2A* mRNA in blood samples from C1D10 were 2.51-fold higher (fold change [FC]; interquartile range [IQR], 1.69–5.05), 1.75 FC (IQR, 1.25–2.07), 1.62 FC (IQR, 1.10–2.01), and 0.73 FC (IQR, 0.62–0.92) over baseline, respectively, consistent with the intended MDM2-antagonist mechanism of action for . The mRNA signature scores significantly differ based on response when measured on C1D2 ($P=0.013$) and on C1D10 ($P=0.01$; Figure S4). The mRNA signature score showed consistence in blood samples and bone marrow aspirate for the same patient at baseline (R=0.50; $P=0.016$; Figure S5). Furthermore, strong concordance between *MDM2*

expressions in 28 patients measured under 2 platforms, microarray and quantitative real-time polymerase chain reaction, was observed (R=0.5; *P*=0.019; Figure S6).

We further evaluated the signature with a pharmacologically optimized next-generation MDM2 antagonist RG7388 using pretreatment specimens from a phase 1 study NP28679 (AML patients with relapsed/refractory disease following induction chemotherapy or unsuitable for standard induction therapy;Table 1 and S4). Twenty-one patients receiving RG7388 in combination with cytarabine (Figure S1) were evaluable. Clinical endpoints were defined with the same criterion to NO21279. Consistent with previous findings, the signature scores, derived from qRT-PCR of *MDM2, XPC, BBC3, CDKN2A* in blood leukaemia samples at baseline, were associated with clinical response (*P*=0.001;Table 1; Figure S2). The signature scores distinguished responders from non-responders with AUC=0.90(95% CI, 0.76–1.00;Table 2); higher than AUCs of *TP53* mutation status or *MDM2* mRNA expression (Table 2). Using a signature score cutoff selected by the Youden index, patients may be discriminated by predicted response prior to the therapy with 100% sensitivity and 71% specificity. Correlation was observed again between signature score and response (P=0.02) in multiple regression with *TP53* mutation status and signature.

In summary, we demonstrate a biological classifier discriminates response broadly to MDM2-antagonist therapy. The level of evidence attained by cell line efficacy modeling and response assessments in trials NO21279 and NP28679 (with MDM2 antagonists RG7112 and RG7388, respectively) adds substantial weight to the validity of this panel.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Kojima K, Konopleva M, Samudio IJ, Shikami M, Cabreira-Hansen M, McQueen T, et al. MDM2 antagonists induce p53-dependent apoptosis in AML: implications for leukaemia therapy. Blood. 2005; 106(9):3150–3159. [PubMed: 16014563]
2. Thompson T, Andreeff M, Studzinski GP, Vassilev LT. 1,25-dihydroxyvitamin D3 enhances the apoptotic activity of MDM2 antagonist nutlin-3a in acute myeloid leukaemia cells expressing wild-type p53. Mol Cancer Ther. 2010; 9(5):1158–1168. [PubMed: 20406950]
3. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukaemia. N Engl J Med. 2013; 368(22):2059–2074. [PubMed: 23634996]
4. Manfredi JJ. The Mdm2-p53 relationship evolves: Mdm2 swings both ways as an oncogene and a tumor suppressor. Genes Dev. 2010; 24(15):1580–1589. [PubMed: 20679392]
5. Shangary S, Wang S. Small-molecule inhibitors of the MDM2-p53 protein-protein interaction to reactivate p53 function: a novel approach for cancer therapy. Annu Rev Pharmacol Toxicol. 2009; 49:223–241. [PubMed: 18834305]

6. Vassilev LT. MDM2 inhibitors for cancer therapy. Trends Mol Med. 2007; 13(1):23–31. [PubMed: 17126603]
7. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science. 2004; 303(5659):844–848. [PubMed: 14704432]
8. Tovar C, Graves B, Packman K, Filipovic Z, Higgins B, Xia M, et al. MDM2 small-molecule antagonist RG7112 activates p53 signaling and regresses human tumors in preclinical cancer models. Cancer Res. 2013; 73(8):2587–2597. [PubMed: 23400593]
9. Ray-Coquard I, Blay JY, Italiano A, Le Cesne A, Penel N, Zhi J, et al. Effect of the MDM2 antagonist RG7112 on the P53 pathway in patients with MDM2-amplified, well-differentiated or dedifferentiated liposarcoma: an exploratory proof-of-mechanism study. Lancet Oncol. 2012; 13(11):1133–1140. [PubMed: 23084521]
10. Chumakov PM. Versatile functions of p53 protein in multicellular organisms. Biochemistry (Mosc). 2007; 72(13):1399–1421. [PubMed: 18282133]

Table 1

Cohort Characteristics of AML Patients in the two clinical trials

NO21279 (N=28)			
	Non-Responder	**Responder**	***P* value**
Sample size	23	5	
Median (IQR) age (years)	60.0 (34.5, 67.0)	58.0 (48.0, 65.0)	0.83[a]
Female, n (%)	7 (44)	3 (60)	0.32[b]
TP53 mutations, n (%)	5 (22)	0	0.51[b]
Median (IQR) mRNA signature score at baseline derived from microarray measurements in blood samples	15.2 (14.8, 15.8)	16.4 (16.0, 16.5)	0.005[a]
NP28679 (N=21)			
	Non-Responder	**Responder**	***P* value**
Sample size	14	7	
Median (IQR) age (years)	64.0 (52.0, 73.5)	70.0 (61.5, 71.5)	0.55[a]
Female, n (%)	7 (50)	4 (57)	1.00[b]
TP53 mutations, n (%)	3 (25)	1 (14)	1.00[b]
Median (IQR) mRNA signature score at baseline derived from RT-PCR meansurements in blood samples	4.0 (3.5, 4.6)	5.2 (5.0, 5.5)	0.001[a]

C1D10, cycle 1 day 10; IQR, interquartile range; MDM2, murine double minute 2; RT-PCR, real-time polymerase chain reaction.

[a] P values are derived by Wilcoxon rank-sum test.

[b] P values are derived by Fisher exact test.

Table 2

Predictions from various predictive biomarkers.

	Oncology Cell Lines Collections[a]	NO21279[b] (derived from blood samples)	NP28679[b] (derived from blood samples)
Score			
AUC (95% CI)	0.92 (0.87–0.97)	0.86 (0.71–1.00)	0.90 (0.76–1.00)
Specificity[c]	0.9	0.65	0.71
Sensitivity[d]	0.87	1	1
TP53			
AUC (95% CI)	0.87 (0.81–0.93)	0.61 (0.52–0.70)	0.56 (0.36–0.74)
Specificity[e]	0.95	0.22	0.25
Sensitivity[f]	0.8	1	0.86
MDM2			
AUC (95% CI)	0.84 (0.77–0.90)	0.60 (0.36–0.83)	0.77 (0.52–1.00)
Specificity[c]	0.75	0.35	0.86
Sensitivity[d]	0.8	1	0.71

AUC, area under the curve; IC_{50}, half maximal inhibitory concentration; *MDM2*, murine double minute 2.

[a] Responders defined as IC_{50} <1 and nonresponders defined as IC_{50} >10 in cell lines.

[b] Responders defined as patients having bone marrow blasts < 5% after treatment and nonresponders defined as patients having bone marrow blasts >= 5% after treatment in NO21279 and NP28679.

[c] Specificity: proportion of nonresponders who had scores or *MDM2* expression lower than the corresponding Youden index.

[d] Sensitivity: proportion of responders who had scores or *MDM2* expression higher than the corresponding Youden index.

[e] Specificity: proportion of nonresponders who had *TP53* mutations.

[f] Sensitivity: proportion of responders who had wild-type *TP53*.