

5-1-2016

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

Bernhard Reis

Lori Jukofsky

Gong Chen

Giovanni Martinelli

Karen Seiter

New York Medical College

See next page for additional authors

Follow this and additional works at: https://touro scholar.touro.edu/nymc_fac_pubs



Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Reis, B., Jukofsky, L., Chen, G., Martinelli, G., Zhong, H., So, W. V., . . . Pierceall, W. E. (2016). Acute myeloid leukemia patients' clinical response to idasanutlin (RG7388) is associated with pre-treatment MDM2 protein expression in leukemic blasts. *Haematologica*, 101(5), e185-e188. doi:10.3324/haematol.2015.139717

This Letter to the Editor is brought to you for free and open access by the Faculty at Touro Scholar. It has been accepted for inclusion in NYMC Faculty Publications by an authorized administrator of Touro Scholar. For more information, please contact touro.scholar@touro.edu.

Authors

Bernhard Reis, Lori Jukofsky, Gong Chen, Giovanni Martinelli, Karen Seiter, and William Pierceall

Acute myeloid leukemia patients' clinical response to idasanutlin (RG7388) is associated with pre-treatment MDM2 protein expression in leukemic blasts

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 ubiquitination/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 (CD45^{dim} or CD45^{dim}/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 ubiquitination and degradation, thus stabilizing the p53 protein to exert tumor suppressor transcriptional regulation and cause induction of apoptotic pathways.¹ 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^{2,3}

may offer a unique paradigm for targeting a co-opted mechanism by oncology therapeutics.⁴

Previously, MDM2 antagonism efficacy modeling was analyzed using large oncology cell line panels, with resulting multi-marker algorithms associated with response.^{5,6} *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.⁵ 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.⁵ 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.⁷ 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 CD45^{dim} blasts and CD45^{dim}/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

Table 1A. MDM2 Protein Expression in AML Blast Cells and CR Association (Proof of Principle and Validation Sets).

	Proof of concept		Validation	
	Wilcoxon	AUC [95%CI]	Wilcoxon	AUC [95%CI]
CD45 ^{dim}	0.0039	0.73 [0.57, 0.89]	0.0062	0.78 [0.60, 0.95]
CD34 ⁺ /CD45 ^{dim}	0.024	0.68 [0.49, 0.87]	0.0016	0.82 [0.65, 0.99]
CD34 ⁺ /CD45 ^{dim} /CD117 ⁺	0.015	0.70 [0.52, 0.88]	0.00069	0.87 [0.72, 1.01]

Table 1B. MDM2 Protein Expression and CR Association in All Patients and WT-only Subset.

	All Patients		Wild-type patients only	
	Wilcoxon	AUC [95%CI]	Wilcoxon	AUC [95%CI]
CD45 ^{dim}	0.00019	0.74 [0.62, 0.85]	0.00053	0.73 [0.61, 0.86]
CD34 ⁺ /CD45 ^{dim}	0.00046	0.73 [0.61, 0.84]	0.0016	0.71 [0.59, 0.84]
CD34 ⁺ /CD45 ^{dim} /CD117 ⁺	0.00018	0.74 [0.63, 0.86]	0.00054	0.74 [0.61, 0.87]

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 $CD45^{dim}/CD34^{+}$ 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,

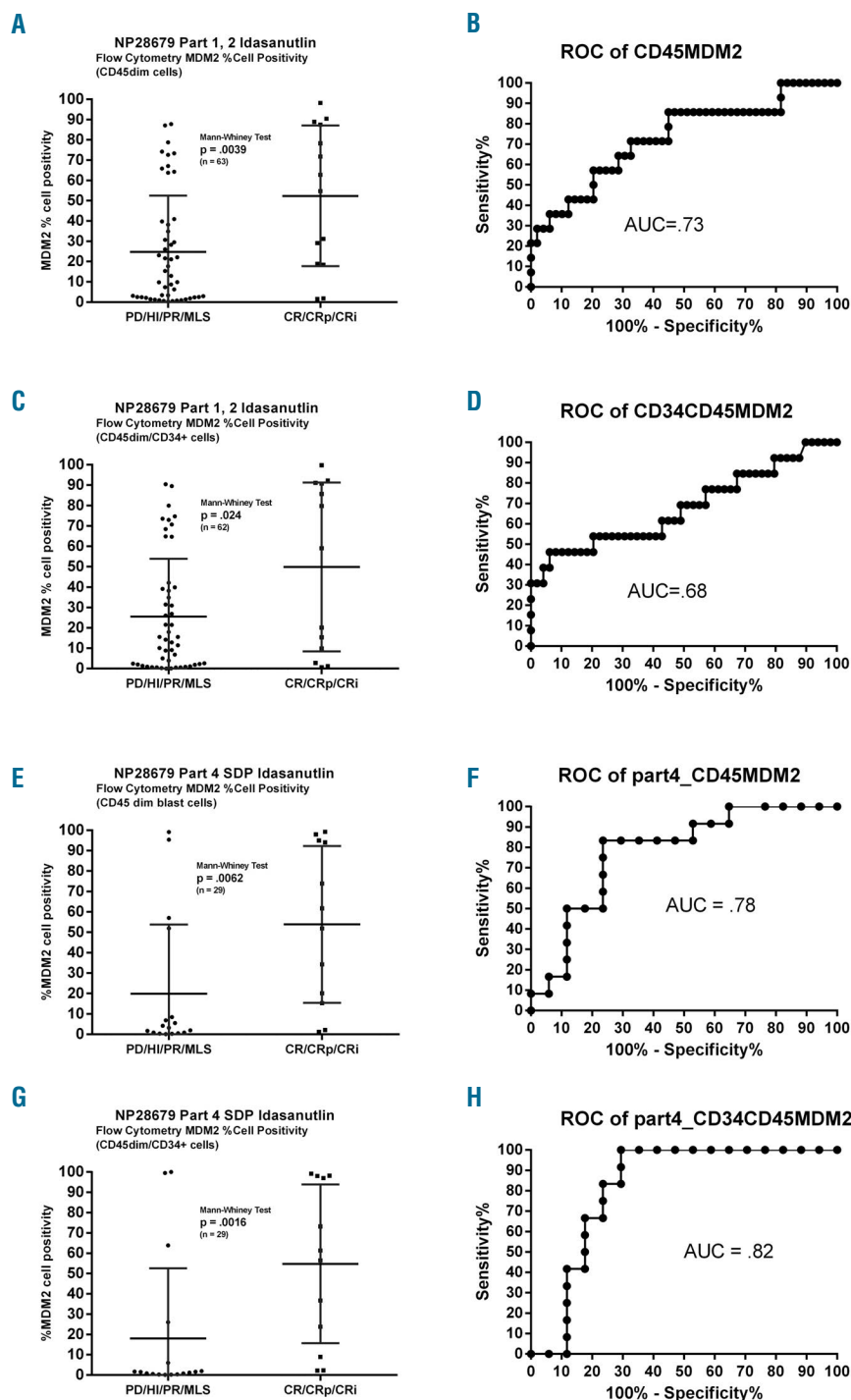


Figure 1. MDM2 Protein Expression in AML Blasts Association with Composite Complete Remission (CRc). Dot plot and AUC depictions for CRc versus non-CR for idasanutlin-based therapy in AML patient blasts (proof of principle and validation sets). (A) Proof of principle dot plot for $CD45^{dim}$ blasts (B) Proof of principle AUC for $CD45^{dim}$ blasts (C) Proof of principle dot plot for $CD45^{dim}/CD34^{+}$ blasts (D) Proof of principle AUC for $CD45^{dim}/CD34^{+}$ blasts. (E) Validation set dot plot for $CD45^{dim}$ blasts (F) Validation set AUC for $CD45^{dim}$ blasts (G) Validation set dot plot for $CD45^{dim}/CD34^{+}$ blasts. (H) Validation set AUC for $CD45^{dim}/CD34^{+}$ blasts.

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 CD45^{dim} 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 CD45^{dim}/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 CD45^{dim} cells (Figure 2 and Table 1B; Wilcoxon $P=0.00019$; AUC, 0.74; 95% CI, 0.62-0.85) and CD45^{dim}/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 CD45^{dim} ($n=73$; $P=0.00053$; AUC, 0.73; 95% CI, 0.61-0.86) and CD45^{dim}/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 CD45^{dim} blasts and the CD45^{dim}/CD34⁺ subpopulation was similar. In additional flow cytometry analyses, we found that clinical response association with CD45^{dim}/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-

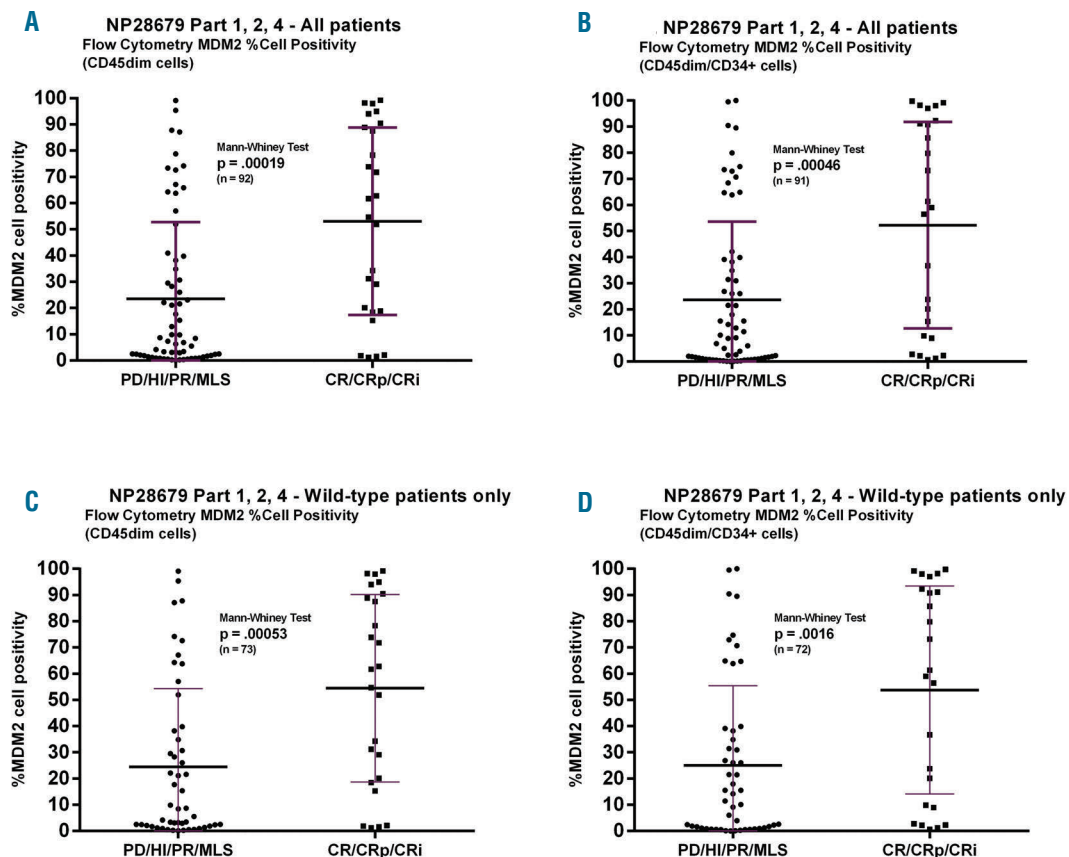


Figure 2. MDM2 Protein Expression and CRc Association in All Patients and WT-only Subset. Dot plot depictions for CRc versus non-CR for idasanutin-based therapy in AML patient blasts for combined proof of principle and validation patient sets; all patients compared to *TP53* WT-only patients. (A) All patients in CD45^{dim} blasts (B) All patients in CD45^{dim}/CD34⁺ patients (C) *TP53* WT patients only in CD45^{dim} blasts (D) *TP53* WT patients only in CD45^{dim}/CD34⁺ blasts.

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,⁸ 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,"⁹ 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 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.

Bernhard Reis,¹ Lori Jukofsky,² Gong Chen,² Giovanni Martinelli,³ Hua Zhong,² W. Venus So,² Michael J. Dickinson,⁴ Mark Drummond,⁵ Sarit Assouline,⁶ Maneja Hashemyan,² Michel Theron,¹ Steven Blotner,² Je-Hwan Lee,⁷ Margaret Kasner,⁸ Sung-Soo Yoon,⁹ Ruediger Rueger,¹⁰ Karen Seiter,¹¹ Steven A. Middleton,² Kevin R. Kelly,¹² Norbert Vey,¹³ Karen Yee,¹⁴ Gwen Nichols,² Lin-Chi Chen,² and William E. Pierceall²

¹Roche Innovation Center Basel, Roche Pharma Research and Early Development, Switzerland; ²Roche Innovation Center New York, Roche Pharma Research and Early Development, NY, USA; ³Seragnoli Institute of Hematology, Bologna University School of Medicine, Italy; ⁴Department of Haematology, Peter MacCallum Cancer Center, Melbourne, VIC, Australia; ⁵Beatson West of Scotland

Cancer Center, Gartnavel General Hospital, Glasgow, United Kingdom; ⁶Division of Hematology, Jewish General Hospital, McGill University, Montreal, QC, Canada; ⁷Department of Hematology, Asian Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; ⁸Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA; ⁹Department of Internal Medicine, Seoul National University School of Medicine, Seoul, South Korea; ¹⁰Roche Innovation Center Penzberg, Roche Pharma Research and Early Development, Germany; ¹¹New York Medical College, Valhalla, NY, USA; ¹²University of Southern California, Norris Comprehensive Cancer Center, Los Angeles, CA, USA; ¹³Hematology, Institut Paoli Calmettes, Marseilles, France; and ¹⁴Princess Margaret Hospital, Toronto, ON, Canada

Acknowledgments: the authors would like to thank Brian Higgins (Roche Innovation Center-New York), Christian Meisel (Roche Innovation Center-Penzberg) and Jeff Venstrom (Genentech) for helpful conversations on data interpretation.

The online version of this letter has a Supplementary Appendix.

Correspondence: william.pierceall@roche.com
doi:10.3324/haematol.2015.139717

Key words: flow cytometry, complementary diagnostics, AML, idasanutlin, MDM2 antagonism.

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

- Vassilev LT, Vu BT, Graves B, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*. 2004;303(5659):844-848.
- Tovar C, Rosinski J, Filipovic Z, et al. Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: implications for therapy. *Proc Natl Acad Sci USA*. 2006;103(6):1888-1893.
- Ray-Coquard I, Blay JY, Italiano A, 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.
- Torti D, Trusolino L. Oncogene addiction as a foundational rationale for targeted anti-cancer therapy: promises and perils. *EMBO Mol Med*. 2011;3(11):623-636.
- Zhong H, Chen G, Jukofsky L, et al. MDM2 antagonist clinical response association with a gene expression signature in acute myeloid leukaemia. *Br J Haematol*. 2015;171(3):432-435.
- Jeay S, Gaulis S, Ferretti S, et al. A distinct p53 target gene set predicts for response to the selective p53-HDM2 inhibitor NVP-CGM097. *eLife*. 2015:e06498.
- Yee K, Martinelli G, Vey N, et al. Phase 1/1b study of RG7388, a potent MDM2 antagonist, in acute myelogenous leukemia (AML) patients. *Blood*. 2014;124(21):116.
- Andreeff M, Kelly KR, Yee K, et al. Results of the phase 1 trial of RG7112, a small molecule MDM2 antagonist in leukemia. *Clin Cancer Res*. 2015 Oct 12. [Epub ahead of print].
- Milne C-P, Bryan C, Garafalo S, McKiernan M. Complementary versus companion diagnostics: apples and oranges? *Biomark Med*. 2015;9(1):25-34.