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

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Supplemental

Methods

Flow cytometry

Cells were stained according to the following procedure: 100 µL of donor whole blood was added to appropriately labeled tubes. Surface markers were subsequently stained by adding antibody fluorochrome conjugates; to facilitate population identification, respective isotype controls were added to control tubes. Tubes were then vortexed and incubated for 20 minutes in the dark at room temperature. After washing with phosphate-buffered saline (PBS) bovine serum albumin (BSA) buffer, tubes were centrifuged and decanted. Two milliliters of prewarmed 1× BD lyse/fix solution was then added and tubes were vortexed and incubated for 15 minutes in a 37°C water bath. After incubation, tubes were centrifuged and the supernatant was decanted and washed with PBS BSA buffer. Then 500 µL of perm buffer III was added and incubated according to the manufacturer's specifications. After washing twice, the appropriate antibodies for intracellular staining or isotype controls were added with PBS/ BSA buffer and vortexed and incubated for 30 minutes in the dark at room temperature. These isotype controls were used to establish background staining and determine MDM2 percent cell positivity. Tubes were washed twice with PBS/ BSA buffer and finally resuspended in 500 µL for acquisition in a FACSCanto II flow cytometer (BD Biosciences, San Jose, CA, USA). A total of 250,000 events were acquired.

Electronic gating

Flow cytometry analysis softwares Winlist and Flowjo were used. Initially, a gate is drawn around all cells with normal size (forwards scatter) and granular characteristics (side scatter): the total nucleated cell gate (TNC; Figure S1). Within this TNC subset, the AML blast population is then identified by drawing a region around cells with dim CD45 expression and low side scatter properties ($CD45^{dim}$ blasts in Figure S1). The blast population can be further refined by gating on CD34-positive or CD34-negative blasts (Figure S1; $CD45^{dim}/CD34^{+}$ or $CD45^{dim}/CD34^{-}$ blasts, respectively). The threshold for CD34

positivity can be identified using an isotype control staining in that respective channel (not shown).

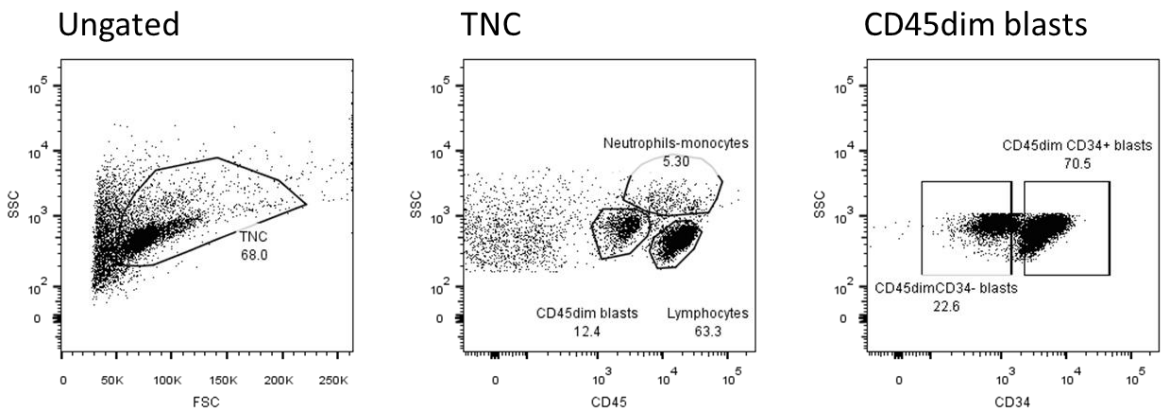

Figure S1. Identification of $CD45^{dim}$/side scatter (SSC) low blasts and $CD34^{+}$ blasts by electronic gating. The $CD45^{dim}$ general blast population and the more refined blasts that additionally express CD34 are displayed in dot plots as shown in Figure S2. The isotype control used instead of MDM2 staining can be used to set the threshold for MDM2 positivity (upper 2 panels in Figure S2.) The staining conditions, including the MDM2 recognizing antibody, are displayed at the bottom of Figure S2. The cell population falling in the regions defined as MDM2 positive is determined by calculating the percentage of MDM2-expressing cells within the respective blast parent population. TNC, total nucleated cell.

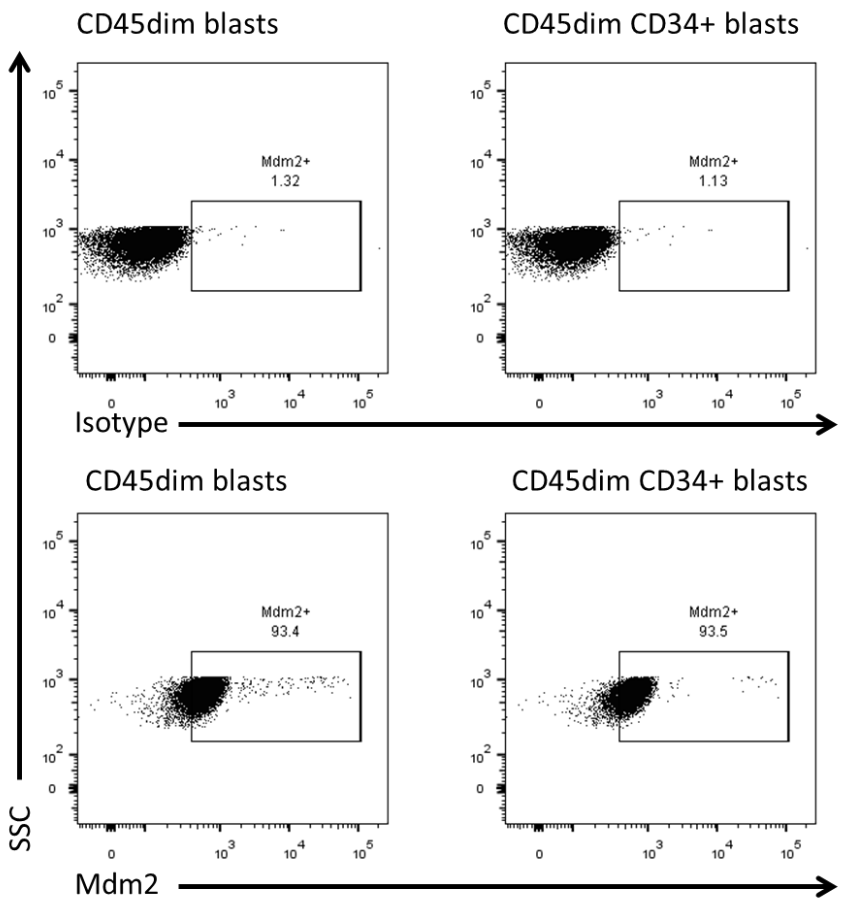

Figure S2. Identification of MDM2-positive cells in the $CD45^{dim}$ blast population and in the $CD45^{dim}/CD34^{+}$ blast population. Isotype controls (upper 2 dot plots) are used to draw the borders for MDM2 threshold positivity. SSC, side scatter.

Table S1. Patient information

Characteristics	CR	Non-CR	All	*P* value
No. of patients	26	66	92	NA
Age, mean (SD), years	62 (11)	60 (13)	60 (12)	.61
Sex, male (female), %	62 (38)	61 (39)	61 (39)	1
Race, white (nonwhite), %	73 (27)	89 (11)	85 (15)	.98
ECOG PS 2 (1, 0), %	0 (46, 54)	9 (67, 24)	7 (61, 33)	.017
TP53, mutant (wild type), %	4 (96)	26 (74)	2 (80)	.018
Treatment, monotherapy (combination), %	20 (80)	42 (58)	36 (64)	.084

All statistical analyses were conducted with R 3.0.2 software. The Wilcoxon rank-sum test was used to examine marginal association between the clinical responses and continuous variables such as MDM2 flow measurements and age. Fisher exact test was used to examine marginal association between the clinical responses and the categorical variables. Area under the concentration curve of receiver-operating characteristic was calculated to indicate complete remission (CR)/non-CR classification performance, with 95% CI calculated with bootstrapping. Logistic regression was adopted to adjust baseline demographic variables and treatment effects (monotherapy or combination therapy). Vuong test was used to compare models with different MDM2 flow measurements. All reported *P* values are 2 sided (*P* significant < .05). ECOG PS, Eastern Cooperative Oncology Group performance status; NA, not applicable.