

LETTER TO THE EDITOR

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# Venetoclax and Daratumumab combination treatment demonstrates pre-clinical efficacy in mouse models of Acute Myeloid Leukemia

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## Abstract

Acute myeloid leukemia (AML) remains an incurable malignancy despite recent advances in treatment. Recently a number of new therapies have emerged for the treatment of AML which target BCL-2 or the membrane receptor CD38. Here, we show that treatment with Venetoclax and Daratumumab combination resulted in a slower tumor progression and a reduced leukemia growth both in vitro and in vivo. These data provide evidence for clinical evaluation of Venetoclax and Daratumumab combination in the treatment of AML.

To the editor,

Acute Myeloid Leukemia (AML) is associated with poor prognosis and high mortality causing around 85,000 global deaths per year [1]. Current standard treatment for AML is poorly tolerated and most therapy is not curative for patients. The intensive nature of proficient therapeutics for AML are more suited towards younger patients, which is not the typical age-group for this disease. This results in limited effective treatment options, highlighting the need for more targeted, well-tolerated treatments.

Recently numerous novel therapies have emerged for treatment of AML. The BCL-2 family proteins are important in regulating intrinsic apoptotic processes [2]. Suppression of mitochondrial-mediated apoptosis by BCL-2 overexpression is a hallmark of AML progression, often correlated with a poor response to cytotoxic treatment [3, 4]. Recently, clinical trials using BCL-2 inhibition by the BH3 mimetic Venetoclax has been shown to be effective in

promoting caspase-dependent AML cell death [5], leading to Venetoclax receiving FDA approval for treatment of AML in combination with low dose cytarabine, decitabine or the hypomethylating azacytidine [6]. This is an important milestone for AML treatment, as no curative therapies have shown to be clinically effective at treating this disease.

Unfortunately, AML resistance to Venetoclax is common, therefore, numerous studies combining Venetoclax with other therapies are being trialed [6, 7]. Our group and others have reported CD38 is overexpressed in AML and CD38 inhibition using Daratumumab is effective in preclinical AML models [8, 9]. CD38 is a transmembrane glycoprotein expressed on many hematopoietic cells [10]. In our recent study we demonstrated that Daratumumab works by inhibiting mitochondrial transfer from mesenchymal stromal cell (MSC) to AML blasts in the bone marrow (BM). Here we hypothesized that inhibiting the CD38 using daratumumab, in combination with BCL-2 inhibition using Venetoclax, could be a strategy to improve overall survival of AML patients.

To investigate BCL-2 inhibition in combination with CD38 inhibition in AML we used primary AML blasts

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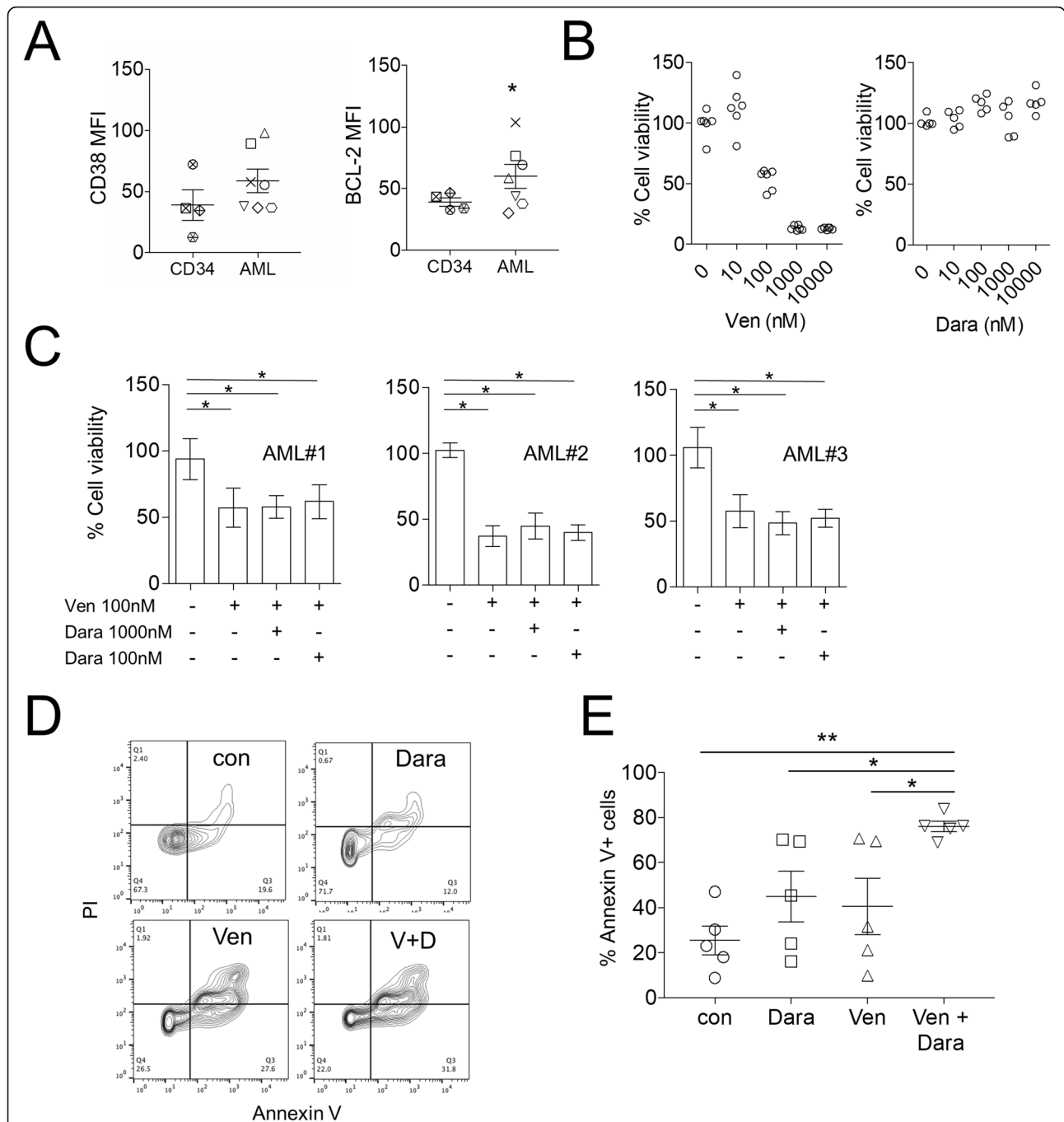
isolated from patients BM (Table 1). CD38 and BCL-2 expression on isolated AML and non-malignant control CD34 cells were measured by flow cytometry (Fig. 1 a and supplementary Figure 1). Cell viability was assessed using various doses of Venetoclax or Daratumumab and showed that 100nM Venetoclax alone inhibited AML cell viability (Fig. 1b). Daratumumab alone had no effect on cell viability at any concentration used (Fig. 1b). Furthermore, in combination with Venetoclax, Daratumumab had no additive effect on AML survival (Fig. 1 c). Since AML is highly reliant on the BM microenvironment, we used an *in vitro* coculture system, AML was cultured with MSC with either Venetoclax alone, Daratumumab alone, or Venetoclax and Daratumumab combination for 24 h. AML cells were then stained with AnnexinV-FITC/PI and analyzed using flow cytometry. Cells treated with Venetoclax and

Daratumumab combination had significantly higher apoptosis compared to untreated AML cells (Fig. 1d and e). Treatment with Venetoclax or Daratumumab alone had no significant effect on AML apoptosis when cultured under these conditions, suggesting that these targeted therapies are working through different pathways. Together, these results highlight the need to study the effectiveness of Venetoclax and Daratumumab combination in *in vivo* models of AML.

To do this we used a NOD.Cg-Prkdcscid IL2rgtm1Wjl/SzJ (NSG) xenograft mouse model of AML whereby on day 1 we transplanted  $0.5 \times 10^6$  MV411 cells or patient derived AML tagged with a luciferase construct into the tail vein of NSG mice. On day 7 we analyzed NSG mice for AML engraftment. Mice were split into treatment groups including vehicle control (PBS),

**Table 1** AML diagnostic information

Number	Age	Sex	Diagnosis	Molecular Karyotype	Molecular genetics	CD38 MFI	BCL-2 MFI	symbol
1	61	M	Acute myeloid leukaemia with a monocytic component	46XY	FLT3 internal tandem duplication; NPM1 exon 12 mutation : CEBPA mutation : Pathogenic variants in DNMT3A and NPM1			
2	74	M	Acute myeloid leukaemia with myelodysplasia related changes	Complex	No mutations in Flt3, NPM1 exon 12 or CEBPA	42	43	⬡
3	68	M	Acute myeloid leukaemia with two FLT-3 ITDs detected with a high allelic ratio (FLT3-ITDhigh).	46XY	FLT3-ITDhigh; no mutations in NPM1 exon 12 or CEBPA	55	73	○
4	59	M	Acute myeloid leukaemia with myelodysplasia related changes	Complex	No mutations in Flt3, NPM1 exon 12 or CEBPA	58	102	X
5	54	F	Acute myeloid leukaemia with MLL (11q23) rearrangement	Tetrasomy 8 and t(9;11)(p21.3;q23.3); KMT2A-MLL3	No mutations in Flt3			
6	59	M	Relapsed Relapse of Acute myeloid leukaemia, with bi-allelic CEBPA mutations	Complex	Pathogenic variants detected in DNMT3A, GATA2 and CEBPA(two variants)	92	79	□
7	55	F	Acute myeloid leukaemia with myelodysplasia related changes	Monosomy 7; Trisomy 8	Pathogenic variants in RUNX1, SRSF2, ASXL1 and TET2; No mutations in Flt3, NPM1 exon 12 or CEBPA	98	54	△
8	75	F	Acute myeloid leukaemia with myelodysplasia related changes	Trisomy 8	No mutations in Flt3, NPM1 exon 12 or CEBPA	43	48	▽
9	37	M	Acute myeloid leukaemia without maturation	46XY	No mutations in Flt3, NPM1 exon 12 or CEBPA			

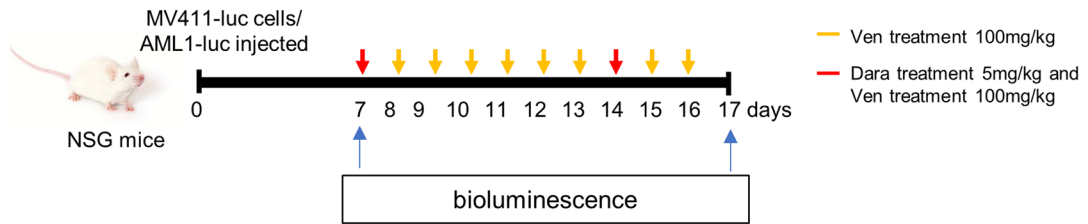


**Fig. 1** Venetoclax and Daratumumab inhibit AML cell growth. **a** Primary AML and CD34 + flow cytometry analysis for CD38 and BCL-2 expression as assessed by MFI ( $n = 7$ ). **b** Primary AML (#1–6) were cultured together for 24 h in the presence of varying concentrations of Venetoclax alone or Daratumumab alone. Cell viability was assessed by CellTiter-Glo ( $n = 6$ ) and expressed as a percentage compared to control untreated cells. **c** Primary AML were cultured together for 24 h in the presence of Venetoclax and varying concentrations of Daratumumab. Cell viability was assessed by CellTiter-Glo ( $n = 3$ ). **d** and **e** Primary AML (#1, #2, #3, #7 and #9) and MSC cultured together for 24 h in the presence of vehicle, Venetoclax (100nm) alone or Daratumumab (100ng/ml) alone or Venetoclax and Daratumumab in combination. Primary AML were then stained with Annexin V/PI, flow cytometry was used to detect cell death ( $n = 5$ ). Statistical tests used were Mann-Whitney U test (A) or Kruskal-Wallis statistical test followed by Dunn’s multiple comparisons (b-e). Data shown are means  $\pm$  SD \* $P < 0.05$  \*\* $P < 0.01$

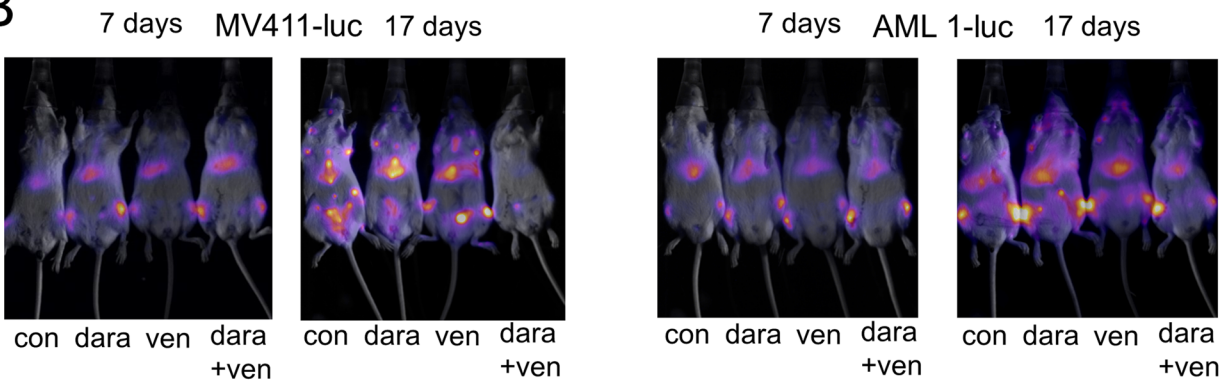
Daratumumab alone on day 7 and 14 by intraperitoneal injection (5 mg/kg), Venetoclax alone by oral gavage (100 mg/kg/day) for 10 days or combination

Daratumumab (5 mg/kg) and Venetoclax (100 mg/kg/day) (Fig. 2 a). The mice were then imaged using live *in vivo* bioluminescence imaging on day 17 to determine

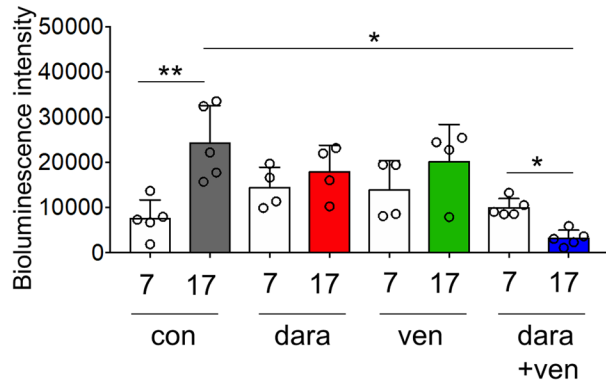
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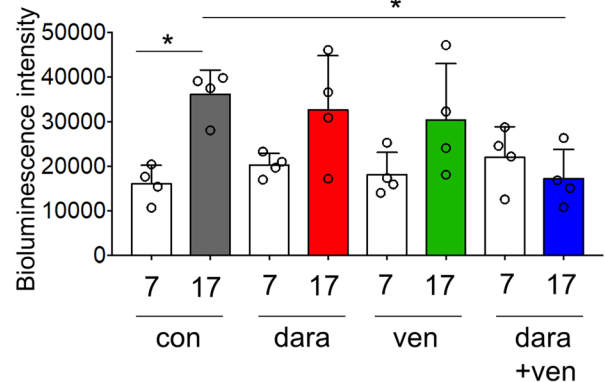
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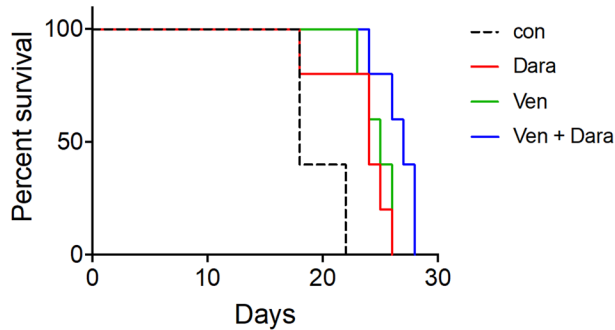
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**D**



**E**



**F**

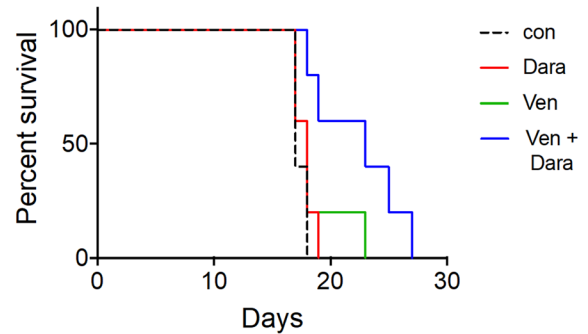


Fig. 2 (See legend on next page.)

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**Fig. 2** Venetoclax and Daratumumab combination inhibits AML disease progression *in vivo*. **a** Schematic of the *in vivo* model used for these experiments. **b**  $0.5 \times 10^6$  MV411 cells or primary AML (#3) were injected into the tail vein of NSG mice. Mice were imaged using bioluminescence at day 7 following injection to confirm tumor engraftment, and then split into four groups. Group 1 received vehicle (PBS), group 2 received Daratumumab (Dara; 5 mg/kg) on day 7 and day 14 by intra-peritoneal (IP) injection, group 3 received Venetoclax (Ven; 100 mg/kg) daily by oral gavage (OG) and group 4 received Venetoclax (Ven; 100 mg/kg) daily by OG and Daratumumab (Dara; 5 mg/kg) on day 7 and day 14 by IP. Mice were then imaged using bioluminescence at day 17. **c** and **d** Densitometry of the bioluminescent images shown in (B) was performed to determine differences between vehicle, Daratumumab alone, Venetoclax alone or Daratumumab and Venetoclax treated animals. ( $n > 4$ ). Statistical tests used was Kruskal-Wallis statistical test followed by Dunn's multiple comparisons. Data shown are means  $\pm$  SD \* $P < 0.05$  \*\* $P < 0.01$ . (E and F) Kaplan-Meier survival curves of treatment groups

tumor burden (Fig. 2b). The densitometry measurement of treated animals and survival graphs confirmed combination Daratumumab and Venetoclax was the most effective at reducing tumor burden (Fig. 2 c-f). Given the heterogeneity of AML, one limitation of the study is that only one AML patient sample and one cell line was used in the *in vivo* experiments. Finally, in phase 1/2 clinical trials in relapse/refractory myeloma combining Venetoclax with Daratumumab has a good safety profile [11].

Here, Venetoclax and Daratumumab combination treatment resulted in a reduced leukemia growth *in vitro* and *in vivo*. Our data opens a potential avenue for novel Venetoclax drug combination with Daratumumab and supports further clinical investigation as a therapeutic approach in the treatment AML.

#### Abbreviations

AML: Acute myeloid leukemia; BCL-2: B-cell lymphoma 2; BM: Bone marrow; IP: Intra-peritoneal; MSC: Mesenchymal stromal cells; NSG: NOD.Cg-Prkdcscid IL2rgtm1Wjl/SzJ; PBS: Phosphate buffered saline

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40364-021-00291-y>.

**Additional file 1:**

**Additional file 2:**

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#### Authors' contributions

J.J.M., K.M.B and S.A.R designed the research; J.J.M., C.H., A.L, J.A.M., and A.J., performed the research; J.J.M., S.A.R., and C.H. carried out *in vivo* work; K.M.B. and A.C provided essential reagents and knowledge. J.J.M., K.M.B. and S.A.R. wrote the paper. The author(s) read and approved the final manuscript.

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#### Availability of data and materials

All data generated or analysed during this study are included in this published article.

#### Declarations

##### Ethics approval and consent to participate

Primary human tissue was taken following informed consent and under approval from the UK National Health Service Health Research Authority (LRECref07/H0310/146).

All animal work in this study was carried out in accordance with regulations set by the United Kingdom Home Office and the Animal Scientific Procedures Act of 1986.

##### Consent for publication

Informed consent has been obtained from all patients (see above).

##### Competing interests

KMB and SAR receive research funding from Abbvie and Janssen. The remaining author declare no competing financial interests.

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