

Daratumumab inhibits acute myeloid leukemia metabolic capacity by blocking mitochondrial transfer from mesenchymal stromal cells

Acute myeloid leukemia (AML) proliferation is dependent on a complex multi-faceted interplay between the blasts and the bone marrow (BM) microenvironment.¹ We and others have previously demonstrated that functional mitochondria are transferred from the mesenchymal stem cells (MSC) to the AML blasts facilitating progression of the disease,^{2,3} in a process which is hijacked from hematopoietic stem cell response to infection.⁴ Clinical observation trials using venetoclax, which targets BCL2 (which in turn is a key regulator of the mitochondrial apoptotic pathway), in combination with hypomethylating agents showed tolerable safety and favorable overall response rate in elderly patients with AML.⁵ Taken together, these data imply that mitochondria represent an attractive and biologically plausible drug target in the treatment of AML.

CD38 is a transmembrane glycoprotein which is expressed on many cells of the hematopoietic system including malignant plasma cells, red blood cells, myeloid cells, lymphoid cells and subsets of leukemia-initiating AML blasts.⁶⁻⁸ Furthermore, we have recently shown that

CD38 mediates pro-tumoral mitochondrial transfer from MSC to malignant plasma cells in myeloma,⁹ which in part explains why daratumumab (an anti-CD38 monoclonal antibody) is clinically effective in treating patients with myeloma.¹⁰ More recently, daratumumab has been shown to have preclinical activity in AML.¹¹ Therefore, taken together, we hypothesize that daratumumab treatment would impair AML metabolic capacity and consequently inhibit tumor proliferation, via a mechanism which blocks mitochondrial transfer from BMSC to the blasts.

Initially, to determine if CD38 inhibition blocks mitochondrial transfer from MSC to AML blasts, we used an *in vitro* co-culture system. MitoTracker Green FM stain (MTG) was used to quantify mitochondria in AML after co-culture with MSC. We incubated both MSC and AML with MTG. The cells were washed twice in phosphate buffered saline (PBS) and incubated for 4 hours (h). The cells were then co-cultured for 24 h with and without daratumumab. Using flow cytometry, AML was shown to have less MTG fluorescence when treated with daratumumab (Figure 1A-C). Figure 1D shows the presence of mouse mtDNA in human AML after co-culture with mouse MSC, and the transfer of mouse mtDNA to human AML was inhibited by the addition of daratu-

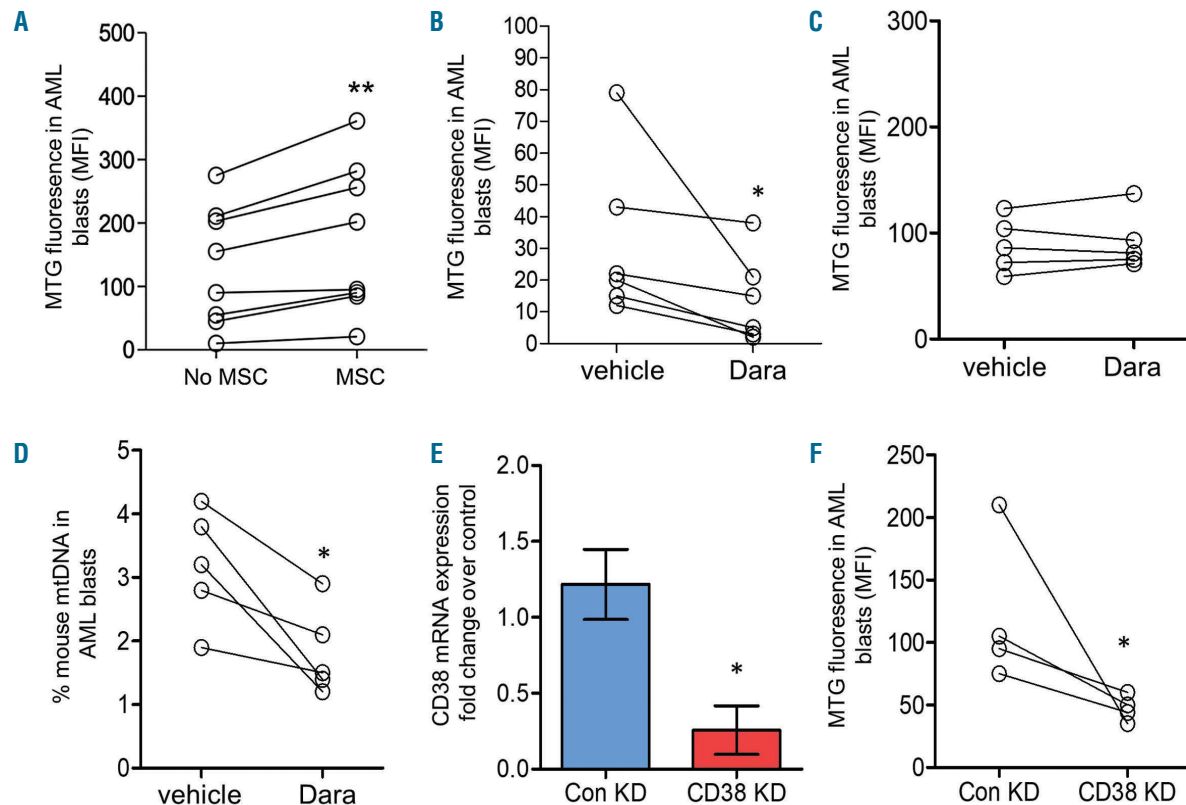


Figure 1. CD38 inhibition blocks mitochondrial transfer from mesenchymal stem cells (MSC) to acute myeloid leukemia (AML) blasts. (A) Primary AML and MSC were pre-stained with MitoTracker green (MTG) fluorescent mitochondrial dye (FM) for 1 hour (h) and then cultured alone or together for 24 h. Flow cytometry was used to detect MTG FM in the AML blasts (n=8). (B) Primary AML and MSC were pre-stained with MTG FM for 1 h and then cultured together for 24 h in the presence of vehicle or daratumumab (Dara) (100 ng/mL). Flow cytometry was used to detect MTG FM in the AML blasts (n=6). (C) Primary AML were pre-stained with MTG FM for 1 h and then treated with vehicle or daratumumab for 24 h (100 ng/mL). Flow cytometry was used to detect MTG FM in the AML blasts (n=5). (D) Human AML was cultured with mouse MSC in the presence of vehicle or daratumumab for 24 h (100 ng/mL) for 24 h. AML were isolated and measured for mouse mitochondrial DNA content. Primary AML were transduced with CD38 targeted shRNA for 48 h. (E) RNA was then extracted and examined for CD38 mRNA expression. (F) AML and MSC were then pre-stained with MTG FM for 1 h and then cultured together for 24 h. Flow cytometry was used to detect MTG FM in the AML blasts (n=4). We used the Mann-Whitney U test and Wilcoxon matched pairs signal rank test to compare results between groups. MFI: mean fluorescence intensity. * $P < 0.05$; ** $P < 0.01$.

mumab. We further showed that knockdown of CD38 inhibited mitochondrial content in AML when cultured with MSC (Figure 1E and F).

We next investigated if daratumumab could inhibit AML disease progression *in vivo*. To do this we used an NSG mouse model of AML whereby on day 1 we transplanted 0.5×10^6 OCI-AML3 cells tagged with a luciferase construct into the tail vein of NSG mice.³ We then treated the NSG animals with either vehicle control (PBS) or daratumumab (5 mg/kg) on days 9 and 16 by intra-peritoneal injection (Figure 2A). The mice were then imaged using bioluminescence on day 21. Figure 2B shows bioluminescence from live *in vivo* imaging. The pre-treatment tumor burden was the same between treatment and control animals on day 9; however, following treatment with

daratumumab, the tumor burden in treated animals was significantly reduced when compared to mice in the vehicle control group. The densitometry measurement of these images are shown in Figure 2C to illustrate the differences between the vehicle control group and daratumumab-treated animals. These data show that there was less tumor-derived bioluminescence intensity in the daratumumab-treated animals when compared to control. Daratumumab-treated animals also had increased overall survival compared to control animals (Figure 2D).

To determine how daratumumab altered the metabolic profile and function of the AML cells *in vivo*, we again transplanted 0.5×10^6 OCI-AML3-luc cells and treated the mice as described in Figure 2. The mice were then sacrificed on day 21. OCI-AML3-luc cells were isolated by cell

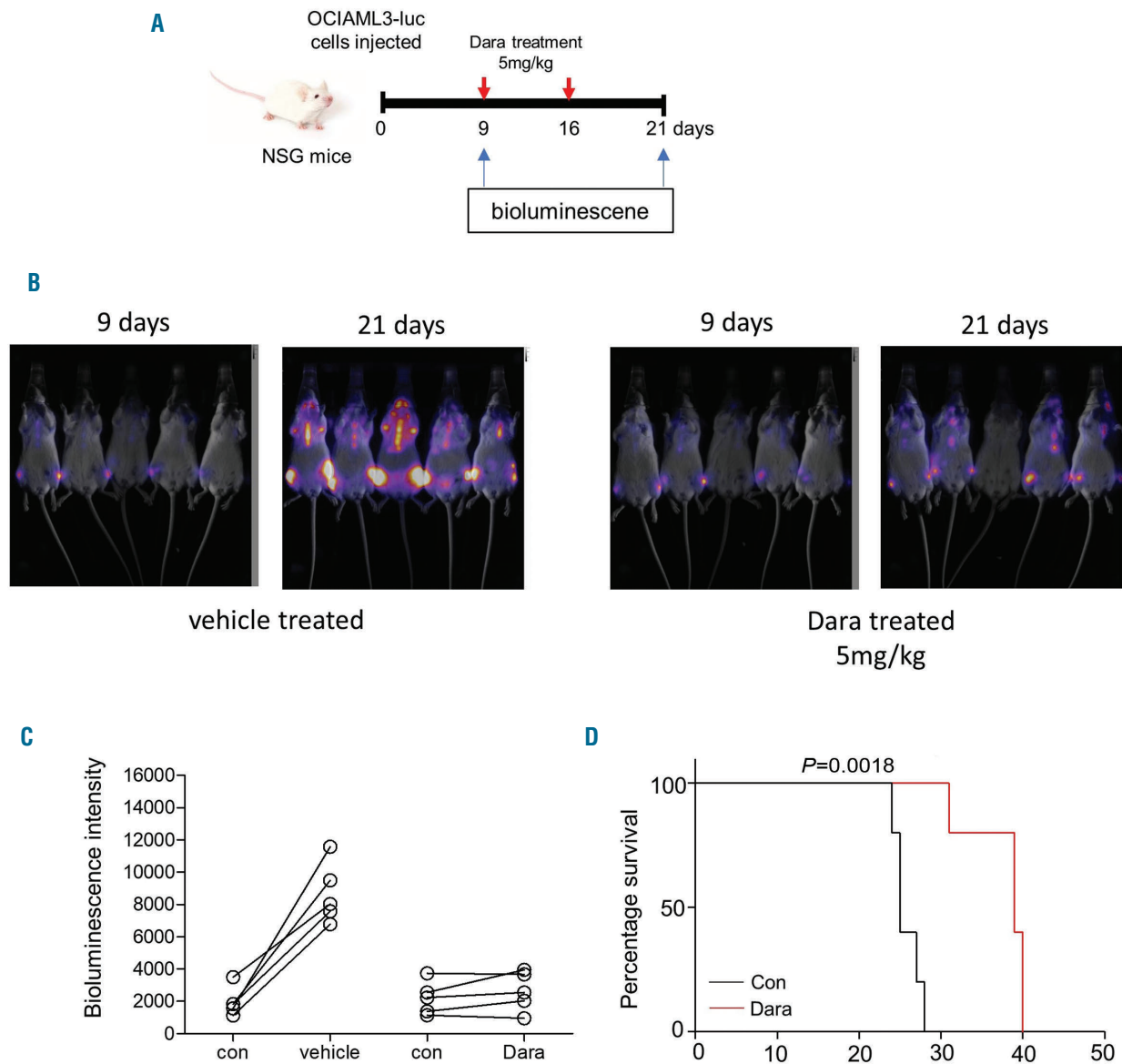


Figure 2. Daratumumab (Dara) inhibits acute myeloid leukemia (AML) disease progression *in vivo*. (A) Schematic of the *in vivo* model used. (B) 0.5×10^6 OCI-AML3-luc cells were injected into the tail vein of NSG mice. Mice were imaged using bioluminescence at day 9 following injection to confirm tumor engraftment, and then split into two groups. Group 1 received vehicle (PBS) and group 2 received Dara (5 mg/kg) on days 9 and 16 by intra-peritoneal (IP) injection. Mice were then imaged using bioluminescence at day 21 and then sacrificed. (C) Densitometry of the bioluminescent images shown in (B) was performed to determine differences between vehicle and Dara-treated animals. (D) In a separate experiment, Kaplan-Meier survival curves were performed for C57BL/6 ($n=5$) mice injected with 0.5×10^6 OCI-AML3-luc and then treated with vehicle or Dara at days 9 and 16 post injection. We used the Mann-Whitney U test to compare results between groups. The Mantel-Cox test was used to analyze Kaplan-Meier survival curves.

sorting from mouse BM and the cells were analyzed *ex vivo* (Figure 3A). The mitochondrial mass within the OCI-AML3-luc cells was not significantly reduced in the animals treated with daratumumab compared to the control animals (Figure 3B). Mitochondrial potential measured by tetramethylrhodamine, methyl ester, perchlorate staining shows that the OCI-AML3-luc cells had a reduced mitochondrial potential in the daratumumab-treated animals compared to the NSG mice who received the control vehicle (Figure 3C). To determine whether daratumumab altered mitochondrial-based metabolism, we analyzed the OCI-AML3-luc cells isolated from the vehicle control and the daratumumab-treated animals and then measured oxygen consumption rate using the Seahorse Cell Mito Stress Test assay. Figure 3D shows decreased mitochondrial respiration was observed in the OCI-AML3-luc cells from daratumumab-treated animals compared to OCI-AML3-luc cells from the control mice.

To investigate if mitochondrial transfer from the BM microenvironment to the AML was altered with daratumumab treatment, the human OCI-AML3-luc cells were isolated from murine BM by cell sorting and analyzed for the presence of mouse mitochondrial DNA within the human OCI-AML3-luc cells by TaqMan real-time polymerase chain reaction. Figure 3E confirms that daratumumab treatment inhibited mouse mitochondrial DNA transfer to human OCI-AML3-luc cells. Together, these results suggest that, *in vivo*, daratumumab treatment causes OCI-AML3-luc cells to decrease in AML mitochondrial mass, and that AML transfer from MSC to AML blasts is reduced and may contribute to the inhibitory functional bio-energetic consequence in AML metabolism in the BM microenvironment.

In conclusion, CD38 inhibition results in reduced mitochondrial transfer from MSC to AML blasts in the BM microenvironment, resulting in a reduction in AML-

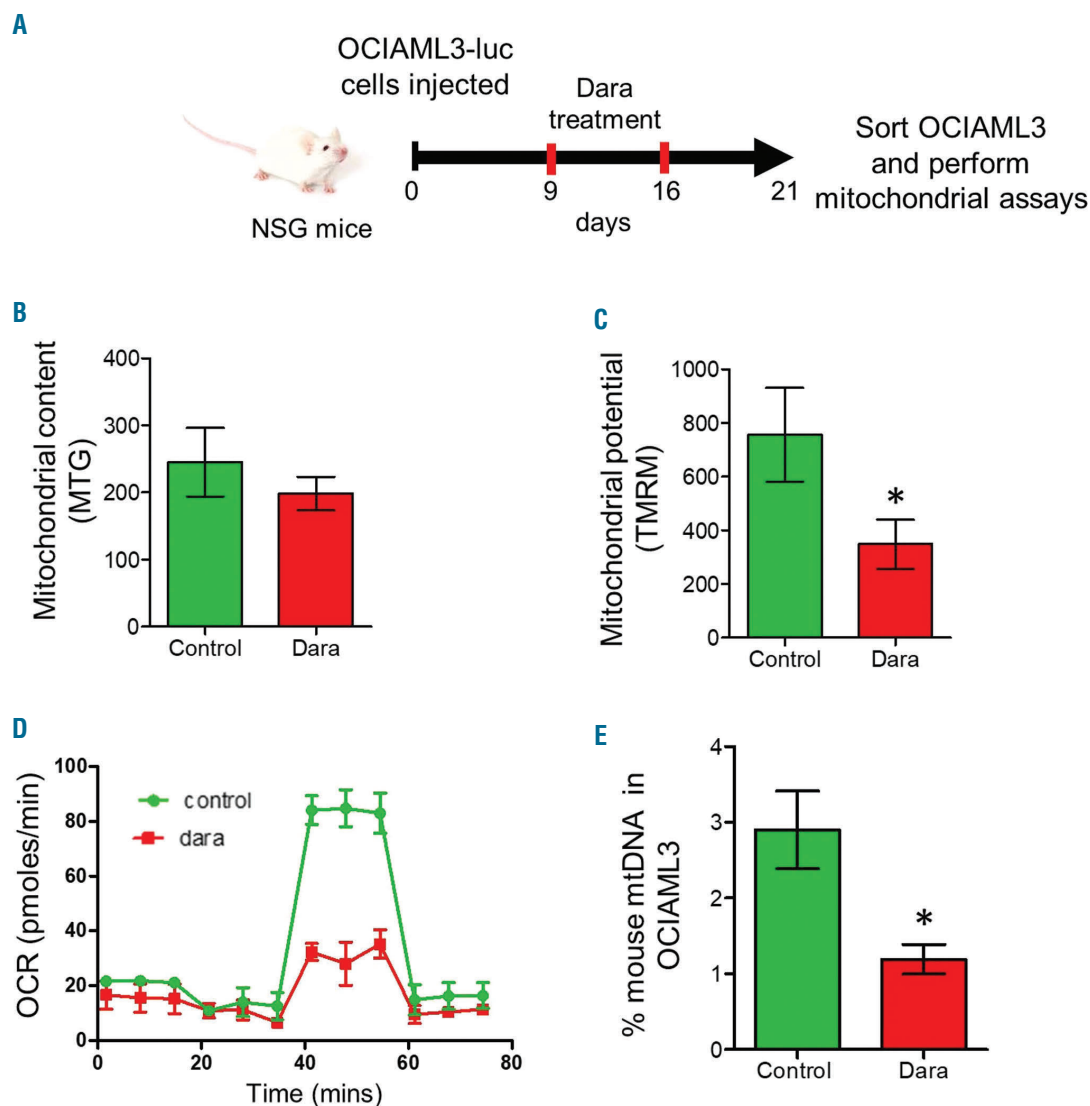


Figure 3. Daratumumab (Dara) alters the metabolic function of acute myeloid leukemia (AML) cells *in vivo*. (A) Schematic of the *in vivo* model for these experiments. 0.5×10^6 OCI-AML3-luc cells were injected into the tail vein of NSG mice. Mice were imaged using bioluminescence at day 9 following injection to confirm tumor engraftment, and then split into two groups. Group 1 received vehicle (PBS) and group 2 received Dara (5 mg/kg) treatment on days 9 and 16 by intraperitoneal (IP) injection. Mice were then sacrificed at day 21 and OCI-AML3-luc cells were isolated by cell sorting from mouse bone marrow and analyzed for (B) mitochondrial content by MitoTracker green staining. (C) Mitochondrial potential by tetramethylrhodamine, methyl ester, perchlorate (TMRM) staining. (D) Oxygen consumption rate (OCR) by Seahorse Cell Mito Stress Test kit. (E) Presence of mouse mitochondrial DNA by real-time polymerase chain reaction. We used the Mann-Whitney U test to compare results between groups. * $P < 0.05$.

derived oxidative phosphorylation and subsequent reduced tumor burden. While it is likely that daratumumab functions through a number of mechanisms of action, here we show in an NSG mouse model lacking functional B cells, T cells and natural killer cells, that inhibition of mitochondrial transfer can be added to the list of mechanisms of action for this drug in AML. These data support the further investigation of daratumumab as a therapeutic approach for the treatment of mitochondrial-dependent tumor growth.

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