Citation: Blood Cancer Journal (2011) 1, e14; doi:10.1038/bcj.2011.13

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## LETTER TO THE EDITOR

# AMD3100 sensitizes acute lymphoblastic leukemia cells to chemotherapy *in vivo*

*Blood Cancer Journal* (2011) **1,** e14; doi:10.1038/bcj.2011.13; published online 1 April 2011

Despite substantial advances in the treatment of acute lymphoblastic leukemia (ALL) during the past four decades, long-term survival remains at approximately 80% for children and 40% for adults. The lack of efficacy of treatment can be partly attributed to the protection provided to the leukemia cells by the bone marrow microenvironment. SDF-1α (CXCL12) is a cytokine produced by bone marrow stromal cells that stimulates the growth of pre-B cells. Its receptor, CXCR4, is expressed on mature and precursor hematopoietic cells. The CXCL12–CXCR4 interaction was shown to be essential for the earliest stages of B-cell development and is also involved in retaining pre-B cells in the bone marrow. Original interest in CXCR4 from a therapeutic point of view was focused on its role as a co-receptor for human immunodeficiency virus, but drugs that block CXCR4, such as AMD3100, can also be used for hematopoietic stem cell mobilization.[1] Juarez *et al.*[2,3] reported that CXCR4 receptor-binding drugs inhibit CXCR4-mediated functions of pre-B ALL cells *in vitro* and can mobilize transplanted ALL cells into the circulation of mice. However, the effect of combined CXCR4 inhibition with chemotherapy on ALL remains to be determined.

We previously showed that AMD3100 is able to block protection provided by a stromal layer to mouse transgenic Bcr/Abl P190 ALL cells treated with Imatinib.[4] To test whether these results can be extended *in vivo*, we performed fluorescence-activated cell sorting analysis on the peripheral blood of leukemic P190 Bcr/Abl ALL (8093)-transplanted mice before and 2 h after intraperitoneal injection with saline or AMD3100, as it has been shown that a single bolus of AMD3100 can mobilize progenitor cells.[1] Interestingly, there was a marked increase in circulating leukemic CD45.2$^+$ (8093 ALL) cells after injection of AMD3100 (Figure 1a) but not in mobilized CD45.1$^+$ C57BL/6 cells. This lack of effect on endogenous CD45$^+$ cells may be caused by the suppression of normal hematopoiesis by the presence of large numbers of malignant lymphoblasts in the bone marrow at this stage. Using the same model, we also examined whether AMD3100 is able to repeatedly mobilize ALL cells in leukemic mice over a longer period of time. As shown in Figure 1b, AMD3100 was able to mobilize a similarly large number of cells into the circulation at days 1 and 10, indicating a sustained mobilizing effect of this drug. These results are consistent with those found in murine transplant models of acute promyelocytic leukemia and multiple myeloma .[5,6] To investigate whether their mobilization into the peripheral blood will make ALL cells more vulnerable to drug treatment, we next tested the combination effect of AMD3100 and nilotinib (AMN107) *in vivo*. Leukemia cells were allowed to proliferate and generate a substantial tumor burden before the start of treatment with phosphate-buffered saline, nilotinib, AMD3100, or a combination of nilotinib and AMD3100 (Figure 1c). As reported previously,[7] nilotinib treatment prolonged the survival of mice. AMD3100 treatment alone had no significant beneficial or detrimental effect on survival (Figure 1c). Interestingly, combination therapy using AMD3100 and nilotinib led to significantly prolonged survival in this murine leukemia model compared with treatment with only nilotinib ($P<0.05$).

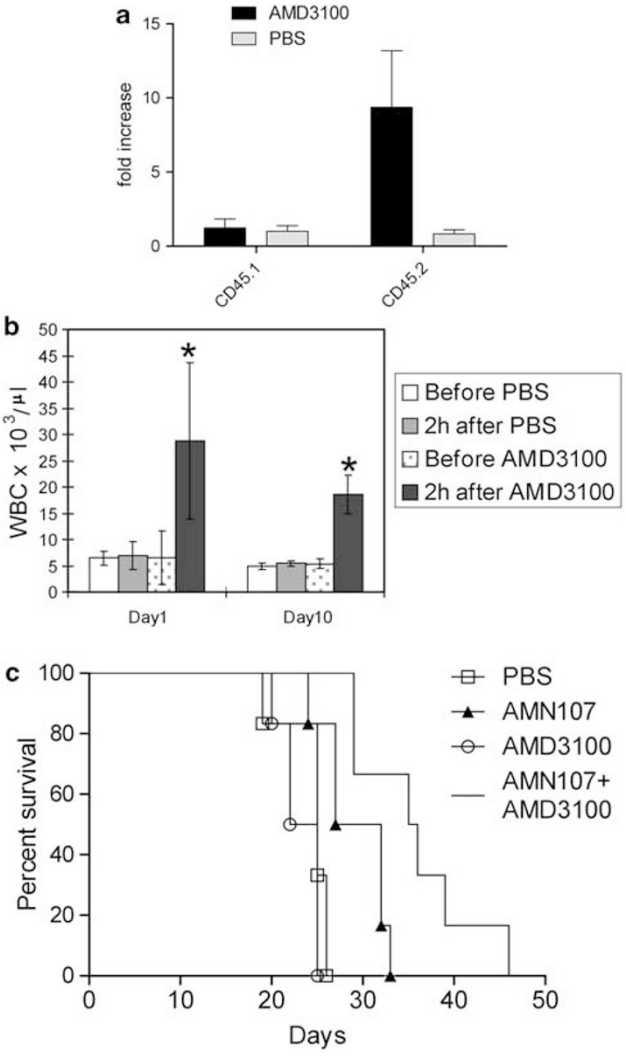


**Figure 1** Combination therapy using AMD3100 and nilotinib prolongs survival of murine ALL transplant recipients. C57Bl mice transplanted with $10^4$ Bcr/Abl P190 ALL 8093 cells were allowed to develop full leukemia within 11–17 days. (**a**) Fold increase 2 h after injection in percentage CD45.1$^+$ or CD45.2$^+$ cells in peripheral blood with respect to percentage before injection with phosphate-buffered saline (PBS) or AMD3100 in the same animal ($n=3$ per group). (**b**) White blood cells on day 17 after transplant (treatment day 1), before and after injection with PBS or before and after injection with 10 mg/kg AMD3100 ($n=3$ mice per group). Mice then received a daily injection with PBS or AMD3100 for 9 more days. On treatment day 10, white blood cells sampling, before and 2 h after injection, was repeated. *$P<0.05$, paired Student's *t*-test. (**c**) Leukemic transplanted mice were treated with PBS, nilotinib/AMN107 (60 mg/kg per d p.o.), AMD3100 (10 mg/kg per d i.p.) or nilotinib + AMD3100 ($n=6$ per group). Nilotinib + AMD3100 versus nilotinib $P=0.047$, log-rank test.

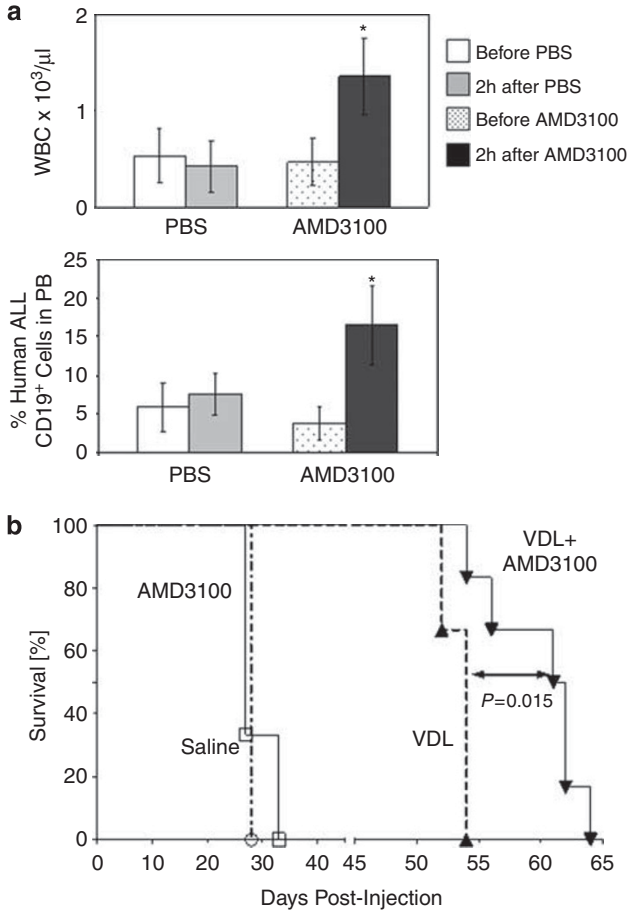


**Figure 2** Preclinical evaluation of AMD3100 as an adjuvant treatment for human drug-resistant ALL. (**a**) Mobilization of human ALL cells. At 12 days after transplant of human pre-B ALL 697 ($6 \times 10^6$ cells/mouse) NOD/SCID/IL2R$\gamma^{-/-}$ mice were injected i.p. with phosphate-buffered saline ($n=3$) or AMD3100 (30 mg/kg) ($n=3$). White blood cells (top) or human CD19$^+$ cells in peripheral blood (bottom) at 2 h after injection. $^*P<0.05$, paired Student's *t*-test. (**b**) Survival of NOD/SCID/IL2R$\gamma^{-/-}$ mice engrafted with primary ALL (US7R) cells and treated with saline (circles, $n=2$), VDL (vincristine 0.5 mg/kg per d, dexamethasone 10.5 mg/kg per d, L-asparaginase 1500 IU/kg per day) (triangles up, $n=3$), AMD3100 (10 mg/kg per day) (squares, $n=3$) or VDL plus AMD3100 (triangle down, $n=6$) for 28 days. AMD3100 was administered via a subcutaneous mini-osmotic pump. VDL + AMD3100 versus VDL, $P=0.015$, log-rank test.

We verified that the AMD3100-mobilized cells included ALL cells by engrafting non-obese diabetic/severe combined immunodeficient mice with the human pre-B ALL cell line 697. As shown in Figure 2a, the increased white blood cells at 2 h after injection with AMD3100 (top) correlated with a significant increase in the peripheral blood of cells expressing human CD19 (bottom), a marker that is highly expressed in this ALL (>99%, data not shown). We next tested the combined treatment of chemotherapy and AMD3100 *in vivo* using primary human US7R, a Philadelphia chromosome-negative ALL. At 12 days after transplant with US7R, mice were started on treatment with phosphate-buffered saline, AMD3100, VDL (Vincristine, Dexamethasone, L-asparaginase) or AMD3100 plus VDL. The untreated control group died rapidly 27 days post-leukemia injection (Figure 2b). Interestingly, mice treated with VDL plus AMD3100 (MST = 61.5 days) survived significantly longer compared with those treated with VDL alone (MST = 54 days; $P=0.015$) or AMD3100 alone (MST = 27 days; $P=0.0022$). Treatment with AMD3100 appeared to be well tolerated, as indicated by continuous weight gain in the treatment groups (data not shown).

The concept of using mobilizing agents to bring ALL cells into the circulation in which they can be more effectively treated with other drugs had not been tested *in vivo*, although Juarez *et al.*[2] did show that AMD3100 enhanced the cytotoxic and anti-proliferative effects of vincristine and dexamethasone in pre-B ALL cells in culture. In AML, AMD3465, a compound related to AMD3100, enhanced the anti-leukemic effects of chemotherapy and sorafenib in mouse transplant models.[8] Use of AMD3100 with Ara-C or with bortezomib in acute promyelocytic leukemia or multiple myeloma also showed that combination treatment sensitized these cancer cells to the therapeutic drug in mouse models.[5,6] Moreover, there are currently ongoing Phase clinical I/II trials (http://clinicaltrials.gov identifier: NCT00512252) for the study of AMD3100 in relapsed or refractory acute myelogenous leukemia in combination with chemotherapy with mitoxantrone, etoposide and cytarabine. In view of the fact, that our studies were performed in models of very advanced primary ALL, in which the animals were allowed to accumulate a substantial tumor burden before treatment was initiated, combined with the fact that the human US7R cells are largely unresponsive to the therapeutic drug combination VDL (results not shown); the effect of AMD3100 combined with a second drug can be regarded as very promising. Thus, clinical trials that test the usefulness of combination treatment with CXCR4 antagonists for therapy of relapsed or high-risk ALL appear to be warranted.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

We thank Markus Müschen for providing patient samples. This work was supported by funding from an RCDA, a Jean Perkins Scholar and a Stop Cancer award (YMK); by the WLBH foundation (YMK, NH, JG) and PHS grant CA090321 (NH).

M Yu[1,4], EJ Gang[2,4], R Parameswaran[1], S Stoddart[1], F Fei[1], S Schmidhuber[1], E Park[2], YT Hsieh[2], AS Yang[3], J Groffen[1,2], N Heisterkamp[1,2,4] and YM Kim[2,4]
[1]*Section of Molecular Carcinogenesis, Division of Hematology/Oncology and The Saban Research Institute of Children's Hospital Los Angeles, Los Angeles, CA, USA;*
[2]*Departments of Pediatrics and Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA and*
[3]*Jane Anne Nohl Division of Hematology, Center for Blood Diseases, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.*
[4]*These authors contributed equally to this work.*
*E-mail: heisterk@hsc.usc.edu*

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