Response to comment on Multifunctional human CD56^{low}CD16^{low} NK cells are the prominent subset in bone marrow of both pediatric healthy donors and leukemic patients

We read with interest the comment by Romee and Miller on our article published in Haematologica[1] demonstrating that there is an increased frequency of the CD56^{low}CD16^{low} natural killer (NK)-cell population in the bone marrow (BM) of children, both healthy subjects and leukemia patients. This NK-cell subset is endowed with higher cytotoxic activity and a similar ability to produce IFNγ as compared to the principal cytokine-producing CD56^{high} NK-cell subset.[2] With regard to the interesting observations raised by Romee and Miller, we think that the CD56^{low}CD16^{low} NK-cell population we described in the BM of leukemia children does not necessarily represent a post-activation NK-cell state, being also present in healthy pediatric donors. However, we cannot completely exclude the possibility that their increased number as compared to those found in healthy donors might also be the result of an *in vivo* post-activation state of NK cells. In accordance with findings reported by Romee *et al.*,[3] Lajoie *et al.*[4] and Grzywacz *et al.*[5] that shedding of CD16 is associated with the activity of A Disintegrin And Metalloproteinase-17 (ADAM17), we have preliminary data indicating that this metalloproteinase is significantly more abundant in the BM plasma of children affected by leukemia, as compared to healthy donors (Figure 1). Unfortunately, at present, the lack of evidence of ADAM17 activity in these samples does not allow us to conclude that the higher number of CD56^{low}CD16^{low} NK cells observed in the BM of leukemia patients, together with their lower expression levels of CD62L, is attributable to increased ADAM17 activity. Our manuscript also shows that CD56^{low}CD16^{low} NK cells are the principal cytotoxic population against both K562 erythroleukemia and autologous leukemia blasts, as evaluated by degranulation assay or ^{51}Cr release assay using as effectors bulk NK cells or sorted NK-cell subsets, respectively. To rule out the possible contribution of CD56^{low}CD16^{high} NK cells in the degranulating activity against K562 exerted by CD56^{low}CD16^{low} NK cells, we performed experiments

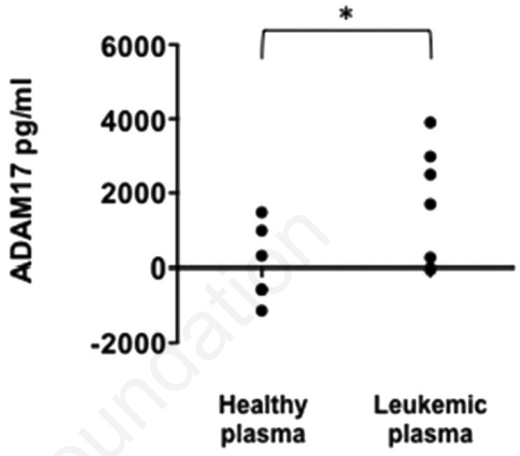

Figure 1. Expression of ADAM17 in bone marrow plasma of pediatric healthy donors and leukemia patients. The plasma of bone marrow aspirate from pediatric healthy donors and leukemia patients at diagnosis was assayed for the expression of ADAM17 by ELISA Kit (R&D system) according to the manufacturer's instruction.*P<0.05. P values were calculated by using t-test.

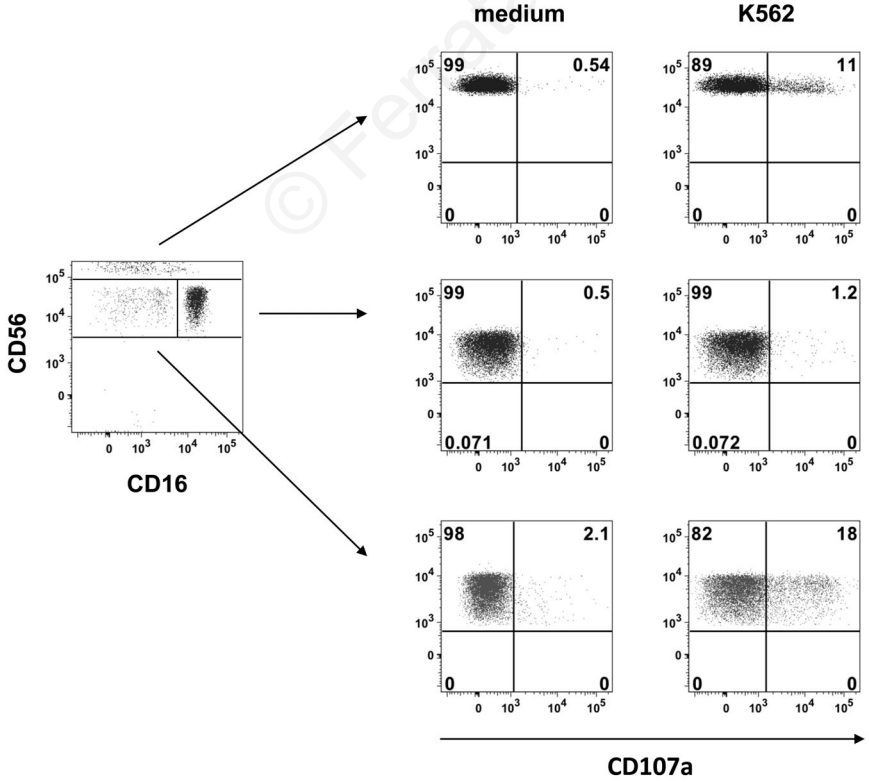

Figure 2. Degranulating activity of peripheral blood (PB) CD56^{high}CD16$^{+/-}$, CD56^{low}CD16^{high} and CD56^{low}CD16^{low} NK-cell subsets. Sorted NK-cell subsets from PB of healthy donors were co-cultured with K562 and degranulation ability of CD56^{high}CD16$^{+/-}$, CD56^{low}CD16^{low} and CD56^{low}CD16^{high}, NK-cell subsets upon 3 h incubation, was assessed by evaluating the percentage of CD107a positive cells. E/T ratio 1:1.

using sorted NK-cell subsets, and we found that CD56l^{low}CD16^{low} NK cells exhibit higher degranulating ability also when sorted NK-cell subsets were used as effector, in the absence of any cytokine pre-activation (Figure 2).

We agree that starting from these observations, it would be very interesting to study the differentiation and maturation state of CD56^{low}CD16^{low} NK cells, as well as the experimental conditions required to selectively expand *in vitro* this NK-cell subset in order to improve NK-cell-based immunotherapy. Experiments are ongoing to clarify these issues.

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Key words: multifunctional human CD56^{low} CD16^{low} natural killer cells, subset, bone marrow, pediatric healthy donors, leukemic patients.

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