Reviewer's report

Title: P-gp activity attenuates AVE9633 and DM4 cytotoxicity in acute myeloid leukemia cells, but is not a major mechanism of chemoresistance

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Reviewer: Charles Dumontet

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The manuscript by Tang et al. reports the study of the impact of three major ABC proteins (Pgp, MRP1, BCRP) on sensitivity of leukemic lines and AML patient samples to the cytotoxicity of an immunoconjugate directed against CD33.

The methodology of this paper is straightforward and this group has broad expertise in the field of ABC proteins in acute myeloid leukemia. Two complementary methods were used both for the analysis of ABC proteins (immunophenotypic detection and functional analysis) and consequences on cell survival (cytotoxicity and apoptosis assays).

A significant part of the paper is devoted to the characterization of ABC expression in the different lines. Curiously K562 variants were used for this study although it does not express CD33 and thus could not be used to evaluate the cytotoxicity of the immunoconjugate.

An interesting conclusion of this paper is the difficulty to correlate results obtained on cell lines in vitro and those obtained with patient samples. As the authors indicate in the discussion this may be due both the differences in level/activity of efflux pumps and differences in levels of sensitivity to the maytansinoid derivative. Patient samples which were resistant to the immunoconjugate were also found to be resistance to DM4.

Major compulsory revisions

1) The title does not reliably translate the main message of this work. “attenuates” is a half-way term which is used here to indicate that the role of Pgp depends on the situation. Actually the authors clearly show that Pgp is a critical factor of resistance in cell lines but does not appear to be clinically relevant. Another title expliciting this difference should be suggested.

Minor essential revisions

review spelling and grammar

Discretionary revisions

1) Insofar as Pgp expression is not correlated with sentivity to the immunoconjugate in AML samples it would be interesting to know whether MRP
or BCRP has an impact in this situation, in spite of the lack of correlation in cell lines. Do the authors have data regarding the MRP and BCRP status in the patient samples?

2) Why did the authors incubate cells for 3 days with DM4 and 4 days with the antibody?

3) CD33 expression is expressed by a majority of blast cells in the samples analysed. However two patients with a low (9 and 36%) expression of CD33 appear to be sensitive to the immunoconjugate in vitro. Could the authors comment on this?

4) In figure 2 HL60 cells with high MRP expression (HL60/Dox) appear to be more sensitive to DM4 and the immunoconjugate than the parental cells. Do the authors have a possible explanation for this?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

Non-financial competing interest:

My hospital laboratory received patient samples during one of the phase I trials of this compound (cf. reference 6) but I have not personally been involved in the development of this immunoconjugate.