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

Version: 1 Date: 20 October 2008

Reviewer: Edward D Ball

Reviewer’s report:

The paper by Tang et al examines the cytotoxicity of an immunoconjugate composed of a humanized monoclonal antibody directed to CD33 linked to the cytotoxic agent DM4 and compares the effects of the immunoconjugate to DM4 alone. The intent of the study was to determine the role of P-gp, MRP1, and BCRP in abrogating the cytotoxicity of the immunoconjugate. Cell lines and primary AML cells were studied. The authors determined that P-gp activity; but not that of MRP1 and BCRP attenuated the cytotoxicity mediated by the immunoconjugate AVE9633 or DM4 in the cell lines. A potent P-gp inhibitor, zosuquidar was able to restore the sensitivity of these cells to the compounds. However, they found that 11 of 26 primary AML samples studied were resistant to AVE9633; but that zosuquidar failed to reestablish drug sensitivity. Resistance to cytotoxicity did not correlate with CD 33 expression or P-gp activity. They conclude that P-gp activity is not a crucial mechanism of chemo-resistance to AVE9633. They further suggest that a conventional anthracycline should be added to ACE9633 in future studies.

Major Compulsory Revisions:

1. The most important issue that needs to be addressed is the nature of the primary AML specimens. The AML patient samples are poorly defined. Were the samples obtained at the time of diagnosis or relapse, or even in remission? The percentage of blasts from the clinical specimens as well as what was actually studied in the mononuclear cell preps should be presented. In other words, what proportion of the cells studied as primary AML cells are actually part of the malignant clone. Moreover, other characteristics of the patients’ cells should be presented such as the FAB classification or the classification in the WHO system. In addition, further information on the nature of the cells, such as any known cytogenetic abnormalities and whether the cells are from primary or secondary AML patients would also lend further credence to the data set.

2. The use of the word “sensibility” is frequently employed when the word should be “sensitivity”.

3. The abstract should define the various acronyms in a better manner.

4. In Materials and Methods, the various cell lines are mentioned in name; but their derivation is not explained. It is more or less perhaps implied that the lines, such as HL60/DNR were developed as chemotherapy resistant lines; but this is
not explained.

Minor Essential Revisions

5. On page 7 under Cell Viability Study, the compounds being studied are referred to as “drugs”. A better term would be compounds, since neither are approved pharmaceuticals at this time.

6. There are numerous typos throughout the paper requiring extensive editing for proper use of the English language.

7. It would have been interesting to see the effects of the AVE9633 compound on CD33 negative cells such as K562s.

8. There are apparent contradictions in the data interpretation. For example, there were responding primary cells in which reversal of P-gp activity did improve activity. It may be true on a statistical basis to say that P-gp activity is not associated with resistance; but it is not true in an individual base. This could be further discussed. The numbers of patient cells that showed weak P-gp activity seems relatively low. What does the literature tell us about the expected expression levels of P-gp in unselected AML patients?

9. It might be of interest to compare the activities of AVE9633 to those of Gemtuzumab Ozogamycin. Given that there is wide variation in AML cell behavior, it would be perhaps more convincing to see discordant activities of one immunoconjugate compared to another and relate these to the P-gp and MRP1 activities.

10. In the various figures, the significance between comparisons would be useful if displayed in the grafts.

11. A final comment is a suggestion to streamline the paper. As written, it is a confusing paper to read as it is replete with considerable data presented in the form of acronyms and ratios and much of which is not statistically significantly different. The paper would be more useful if some of the data were removed from tables and figures and simply mentioned in the text. Also, further attention could be made in explaining the results in the paper in a more concise and cogent manner.

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: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I receive clinical trial funding from Celgene for a study of 5-azacytidine and gemtuzumab ozagamicin in relapsed AML.