Reviewer's report

Title: High class I HDAC activity and expression are associated with RelA/p65 activation in pancreatic cancer in vitro and in vivo.

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Reviewer: Carter Van Waes

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The authors hypothesized that HDAC class I expression would be associated with increased NF-kB activation, which has been shown to promote the malignant phenotype and related to poor outcome in pancreatic carcinoma. They provide evidence that increase in HDAC staining is associated with NF-kB RELA staining, and that HDACIs inhibit NF-kB nuclear staining and binding 72 hours post treatment.

A strength of the study is the characterization of the class I HDACs 1, 2 and 3 and NF-kB in series of 81 patients, and demonstration that HDAC expression is increased in relation to NF-kB, and NF-kB is associated with poor prognosis.

Major issues which decrease enthusiasm would need to be addressed:

1) How do the authors explain the relationships of HDACs to NF-kB staining do not translate to the same relationship of HDAC staining to patient survival as observed between NF-kB and survival? In this regard, the authors appear to have used univariate methods for multiple comparisons, and it is not clear if the associations and conclusions drawn would be the same after appropriate use of multivariate methods. Have they done so? If not, inclusion of a statistician in the analysis and authorship could be helpful.

2) The apparent relationship between HDACs expression and NF-kB activation appears to be opposite the functional role of HDACs found in previous studies by several laboratories (ie Chen et al, Science 2001;293: 1653-7; EMBO J 2002; 21:6539-48). HDACs including HDAC3 found in the authors study have been reported to deacetylate RELA, decreasing its transactivating, DNA binding and nuclear localization. HDACIs have been reported to decrease this, favoring acetylation and increased NF-kB activation (Duan, Mol Cancer Ther, 2007;6:37-50). Is it possible the effects the authors see at relatively late time point of 72 hours reflect effects on histones versus RELA itself, or merely decreased relative protein or degradation of cells undergoing growth arrest or apoptosis after exposure to HDACIs? A time course at 12, 24, 48 and 72 hours with NF-kB luciferase reporter and westerns showing total and acetylation of nuclear p65 and an appropriate histone could elucidate functional and biochemical effects.

3) Along this line, IHC of serial sections of same area within tumors with weak, partial or strong staining with HDACs and relationship to cytoplasmic versus
nuclear RELA and a regression analysis of these from the series could help show such a relationship is dominant in tumors in situ.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I have no competing interests.