Answer to reviewer's comments:

Reviewer 1

It is a great job! The authors concluded the role of Histone post-translational modifications in the diagnosis, treatment and prognosis of cancer and brought a excellent review to me. There are some slight mistakes.

1. When you use abbreviate, you need spell out an abbreviation in full or define it the first time you use it in the main body of the text. For example, PTM (page 4), hMOF (page 7), DMFS (page 13).

A. Authors are grateful to reviewer for pointing out the mistake. All the full forms have been mentioned at the appropriate place in the edited manuscript.

2. Page 9, "Therefore, at one side there is need to improvise techniques and on the other hand discovery of new markers are of immense importance." The word "improvise" should be "improve"? The word "are" should be "is"

A. The grammatical error has been corrected.

3. Page 11, "However, in patients with acute myeloid leukaemia decrease in H3K9me3 found to be associated with better prognosis[74]." The "found" should be "has been found"

A. The grammatical error has been corrected and is the part of edited manuscript.

4. Page 12, "On the other hand in case of breast, prostate, ovarian and pancreatic cancers low level of H3K27me3 had significantly shorter overall survival time when compared with those with high H3K27me3 expression" The "low level of H3K27me3" should be "patients with low level of H3K27me3.

A. Authors are grateful to reviewer for pointing out the mistake. The mistake has been corrected in the edited manuscript.

5. Page 17, "Study involming neuroblastoma xenografts also demonstrated its antitumor activity" The "involming" should be "involving".

A. The spelling mistake has been corrected in the manuscript.

6. Page18, "Increase in the concentration serum nucleosomes have been shown at 24–72 hr after the first application of chemotherapy and 6–24 hr after the start of radiotherapy". The "have" should be "has".

A. The grammatical error has been corrected and is the part of edited manuscript.

7. Page 19, "all the organisms studied so far (from yeast to man) has bought to light the importance of chromatin environment especially histone PTMs in development and disease". The "has "should be "have"

A. The grammatical error has been corrected and is the part of edited manuscript.

8. There are significant difference of histone PTM level between normal and cancer tissues, but are there difference between benign lesion and malignant tissue? In other words, in the progression from normal cells to dysplasia and to cancer, even to metastasis, are there difference?

A. The authors appreciate the query of the reviewer. Tissue microarrays done to compare the levels of H2B ub1 levels in normal mammary epithelial tissue as well as benign, malignant, and metastatic breast cancer samples have clearly shown sequential decrease in H2B mono-ubiquitination with breast cancer progression and metastasis in comparison with normal epithelia.
This is now the part of manuscript in Page 7.

9. In your review, H3K27me3 has been evaluated as a prognostic factor in different cancers, however, in some cancer, it is a factor correlated with poor prognosis, but in other cancer, it is a predictor of better survival. The results are perplexing. Then what cause the perplexing results, histological differences or other reasons?

A. Histone modifications like H3K27me3 have indeed showed perplexing results when analysed with respect to various cancers. This can be attributed to tissue type, and indeed histone PTMs are known to be showing their abundance in a tissue specific manner. This might be as because many writers and erasers utilize co-factors or substrates like acetyl CoA, SAM, NAD+, FAD+ or ATP which are crucial metabolites in core pathways of intermediary metabolism. As because the cellular concentrations of these metabolites fluctuate as the metabolic status of the cell changes, the activity of these enzymes gets affected thus the histone PTMs.
This is now the part of manuscript in Page 19.

Reviewer 2

The manuscript is a review on the complex field of Epigenetics, focused mainly on histone modifications. In my opinion, there is a need to reorganize some parts of the paper so that it is understandable for people not directly working in the field. In this line, a consistent use of abbreviations would be of help. Mention to new histone modifications recently reported should be included, as well as, the relationship of PTMs and intermediary metabolism.

1. Please, include the appropriate reference for the "HIstome database" in addition to the link already provided (page 4, line 12).

A. Authors are grateful to reviewer for pointing out the mistake. The reference has been added to the manuscript.

2. Figure 1 legend, the diagram only shows some examples of modifications affecting histones. Please, modify the last sentence of the legend accordingly

A. Authors thank the reviewers for pointing out the mistake. The legend has been corrected to The diagram indicates **some** modifications at specific residues: M = methylation, A = acetylation, P = phosphorylation

3. Figure 2 legend. The term histone methyltransferases also includes certain protein arginine methyltransferases, since histones are methylated on lysine and arginine residues. Hence, in my opinion, the legend should be modified to clarify this point. Moreover, I think that vertical columns highlighting each, phosphorylation, acetylation or methylation related proteins among writers, readers and erasers would aid in understanding the organization of the scheme

A. The legend of figure 2 has been corrected and it is now part of the edited manuscript.

4. Page 5, 1st paragraph. Several of the mentioned histone modifications can appear in several degrees, for example mono-, di- or trimethylations can occur. This facts is not explained, neither are the abbreviations commonly used (i.e. me, me2, me3) and that appear later in the text.

A. The authors appreciate the reviewer for correcting us. As pointed out "These histone modifications occur at several degrees for example methylation can be of monomethyl (me), dimethyl (me2) and trimethyl (me3)" –this sentence has been included in the manuscript on Page 5, 1st paragraph.

5. Page 6, 1st paragraph refers to the existence of histone variants, which in my opinion should be introduced earlier in the text (end of page 4), before going into the description of post-translational modifications

A. We agree with the reviewer, and hence the histone variants have been now moved to Page 4 last paragraph.

6. Page 6, end of 1st paragraph. The authors explain the existence of crosstalk between several types of PTMs. However, no example is provided of this mechanism.

A. The authors thank reviewer for pointing it out. Hence the below sentence has been added

For example, acetylation of H3K18 and H3K23 by CBP (CREB binding protein) can promote the methylation of H3R17 by CARM1 (Coactivator-Associated Arginine Methyltransferase 1), resulting in activation of estrogen-responsive genes.

7. A list of abbreviation should be provided, and the abbreviations should be consistently used in the text (i.e. phosphorylation appears as P or ph)

A. As throughout the manuscript only Acetylation, Methylation or Phosphorylation is discussed majorly, list of abbreviation has not been included. However as pointed by the reviewer, all the abbreviations have been corrected for consistency throughout the manuscript.

8. No mention is made to the supply of substrates needed to establish the different histone PTMs. Please, see reviews on that subject by: Gut & Verdin (2013) Nature 502, 489-98, Gibson & Kraus. (2011) Mol Cell 41, 497-9, Martinez-Pastor et al. (2013) Cancer Discovery 3, 497-501, LU & Thompson (2012) Cell Metabolism 16, 9-17, etc.

A. We appreciate the suggestion of reviewer and changes have been incorporated.
Many writers and erasers utilize co-factors or substrates like acetyl CoA, SAM, NAD+, FAD+ or ATP which are crucial metabolites in core pathways of intermediary metabolism. As, the cellular concentrations of these metabolites fluctuate with the metabolic status of the cell, the activity of enzymes gets affected and thus the histone PTMs.
Now this is part of manuscript in Page 19.

9. Page 9, line 1. It is stated "there is a need to improvise techniques", I guess authors mean to improve techniques. Otherwise, please explain

A. The grammatical error has been corrected and is the part of edited manuscript.

10. Recently, histone homocysteinylation has been described as a new PTM occurring in these proteins. The authors did not mention these new PTM in the text.

A. We appreciate the suggestion of reviewer, and so the changes have been made in the manuscript on page 5.

11. In my opinion, the last column in Table 1 should only state the confirmed use of the PTMs listed. Moreover, it would be better shown as three independent columns one for each diagnosis, prognosis and treatment (if any).

A. We appreciate the concern of reviewers, all the PTMs listed have been verified for their use, but however in clinics to date the markers are not in confirmed use. And as there are not many PTMs which can be differentiated as per disease diagnosis, prognosis and treatment they have been included as a single column.

12. Last section on cancer treatment, requires reorganization, it is possible that inclusion of subheadings would be of help. A table with most promising treatments could be useful to complete this section.

A. As pointed out by the reviewer, subheadings have been included for this section of manuscript. As single treatment modality is not used in clinics but

rather a combinatorial approach is used, so we believe inclusion of table in this section might not be of much use.

Reviewer 3

In this review, Khan et al. investigate how histone modifications are involved in cancer diagnosis, prognosis and treatment. Overall, the work appears reasonable and useful. Thus, it is deserved to be published in this journal.

Some minor points:

1. Unify the symbol of Ac & ac; Me & me throughout the paper.

A. As pointed out by the reviewer, all the symbols have been unified.

2. In Table 1, it seems missing "reader" part.

A. We appreciate the reviewer for pointing it out, but as the reader molecules for each PTM are multiple, and inclusion of it will make already multidimensional table more complex.

3. Also in Table 1, suggest to add a references column for convenient checking from cited 137 references

A. Indeed addition of references will be good, but as the table itself is multidimensional including PTMs, writers, erasers, function and their use in clinics. Addition of references will lead to crowding of the table and increase its complexity.

Comment of Journal's Chief Editor:

The review has been improved in general. However, there are still a number of small errors (eg page 12, H2AX, is it a gamma?). This reflects the lack of care of the authors to details. As a result, the manuscript is still need further improvement.

Answer to the comment:

We thank the Chief Editor of the journal to point out the small errors which were left behind in last submitted version of the manuscript. In the submitted improved version we have corrected all the errors which we could identify. We also would like to point out that all H2AX is gamma H2AX and we have now used a new and more proper symbol for gamma (γ) to avoid any confusion. The additional minor corrections are in 'track change mode' in the word file and needs to be accepted by the Chief Editor before sending for publication. The new improved version of manuscript will satisfy the Chief Editor's concern.