Ive read through. This will not be hard to revise.

Most is more analysis for the informatics. Sevin, can you list out what you can do in response to the informatics analysis comments.

For the cd24 comment…we can easily argue that the point is not to determine the function of cd24.

For the comments about spike in…..we don't need to do anything…..the histone mark increases are indeed locus specific.
We just need to say and show that.

For the comment on whether the astrocytes look like tumors….we can cite our old paper. And perhaps supplement with a few analyses like you've done for your original nature paper….but just a few items…perhaps heatmap, pathway analysis…just like before

All in all, not to bad

Tim

From: Chan, Timothy/Radiation Oncology
Sent: Wednesday, December 21, 2016 4:00 PM
To: 'Sevin Turcan'; Wang, Yuxiang/Sloan Kettering Institute
Subject: FW: Decision on Nature Genetics submission NG-A44722

Hi Sevin and Yuxiang,

Here are the comments on our IDH paper. Seems like they are positive and are inviting a revision.

Lets read through and decide on how to address the points.

Thanks
Tim

From: brooke.laflamme@us.nature.com
[mailto:brooke.laflamme@us.nature.com]
Sent: Wednesday, December 21, 2016 3:55 PM
To: Chan, Timothy/Radiation Oncology
Subject: Decision on Nature Genetics submission NG-A44722

21st Dec 2016

Dear Tim,

Thank you for your patience while we awaited the referee comments. Your Article, "Mutant IDH1-Dependent Chromatin State Reprogramming, Reversibility, and Persistence" has now been seen by 3 referees. You will see from their comments copied below that while they find your work of considerable potential interest, they have raised quite substantial concerns that must be addressed. In light of these comments, we cannot accept the manuscript for publication, but would be very interested in considering a revised version that addresses these serious concerns.

We hope you will find the referees' comments useful as you decide how to proceed. If you wish to submit a substantially revised manuscript, please bear in mind that we will be reluctant to approach the referees again in the absence of major revisions. My editorial comments are included in the attached document, which you should use to prepare your point-by-point rebuttal.

If you choose to revise your manuscript taking into account all reviewer and editor comments, please highlight all changes in the manuscript text file.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

If revising your manuscript:

* Include the attached “Response to referees” document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

* If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions, avaliable here. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

You may use the link below to submit your revised manuscript and related files:

http://mts-ng.nature.com/cgi-bin/main.plex?el=A1G3BfP1A3nfE6J4A9ftd0OBcrgvWGcpRkclFZV858AZ

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

If you wish to submit a suitably revised manuscript we would hope to receive it within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Genetics or published elsewhere. Should your manuscript be substantially delayed without notifying us in advance and your article is eventually published, the received date would be that of the revised, not the original, version.

Please do not hesitate to contact me if you have any questions or would like to discuss the required revisions further.

Thank you for the opportunity to review your work.

Happy Holidays!
Brooke

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Brooke LaFlamme, Ph.D.
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Referee expertise:

Referee #1: cancer epigenomics

Referee #2: cancer genomics

Referee #3: glioma genomics

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
Over 80% of low grade gliomas and secondary glioblastomas harbor mutations in the isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) genes, most commonly affecting the arginine 132 codon. These mutations results in the accumulation of 2-hydroxyglutarate (2HG), an antagonist to DNA demethylases, resulting in the DNA hypermethylated tumors, referred to a CpG Island Methylator Phenotype (CIMP+) tumors. IDH mutant specific chemical inhibitors are available but they only partially revert the CIMP status. To help improve therapy, the current manuscript focuses on two questions, 1- to what extent are IDH mutations affecting chromatin methylation (inclusive of both DNA and histones) and 2- are the changes fully or partially reversible upon IDH1 mutant inhibition? These questions are addressed by profiling chromatin methylation (DNA as well as histone) in combination with gene expression in dox induced ectopic IDH1 mutant (R132H) expressing immortalized human astrocytes (IHA). In addition,
the authors assess the persistence of the IDH1 R132H induced chromatin methylation changes following dox removal (loss of IDH1 mutant expression) over multiple time points. They conclude that the effect on the chromatin induced upon mutant IDH1 expression is dynamic and that loss of mutant IDH1 expression is characterized by reversibility of some chromatin changes and other irreversible alterations, which may account for the partial response under mutant IDH specific inhibitors.

The authors also report a role for mutant IDH1 in 1- increasing the CD24-positive population in IHA, which relates to an increased sphere forming capacity, 2- inducing the expression of specific classes of endogenous retroviral elements, in line with the

enrichment of interferon alpha responsive genes upregulated in IDH1 mutant expressing versus control cells and 3- promoting genomic instability as measured based on copy-number alterations detectable in mutant expressing cells.

Overall, this manuscript addresses critical questions in the field of research that relate to the role of IDH1 mutations on chromatin methylation, inclusive of DNA methylation and histone methylation, in order to delineate new therapeutic opportunities to improve the partial response associated with mutant IDH1 specific chemical inhibitors. However, while the premise and approach are interesting, significant concerns are raised based on the current format of the manuscript. The conclusions based on the reversible versus irreversible nature of mutant IDH1-associated chromatin changes is not well supported by the data and experiments. For instance, the subset of regions/genes designated as ‘persistent/irreversible’ trend towards reversal at P40 after dox removal based on both DNA methylation and gene expression, suggesting that these are just more long-lasting changes as opposed to irreversible changes.

More comprehensive and integrated analyses of chromatin methylation (histone and DNA) with gene expression changes in IDH1 mutant vs wild-type at all time points. Furthermore, the impact/contribution of mutant IDH1 expression on stemness, endogenous retrovirus expression and genomic instability would need to be investigated in greater detail to provide the reader with a clear understanding of the role of mutant IDH1 in these distinct aspects.

Specific points:

Reversible versus irreversible mutant IDH1-associated chromatin changes:

1- Figure 1 provides clear evidence of reversible changes in gene expression following mutant IDH1 expression and upon its withdrawal in IHA (clearly shown in 1F) paralleled to changes in DNA methylation reported in Figure 3 (3F in particular). However, results presented in Figure 2 do not clearly indicate how histone methylations are affected. Panel 2A-C and F do not allow the reader to effectively detect the behavior of each mark. For instance, an increase in peak number reported under one time point may correspond to a single peak from an earlier time point splitting in two or more peaks. The “apparent” increase in H3K9me3 or k36me3 in figure 2C does not clearly indicate if the increase in methylation signal is occurring at all or at a subset of genomic regions (the figure is too small and the representation of each genomic location is too small for adequate reporting). If the increase is taking place at all peaks than this would argue in favor of a technical bias and
spike-in controls would be required. Heatmap representation for these results focused on the union of called peaks (overlapping by at least one basepair) for each histone modification would be welcome. Also, the exclusion of H3K4me1 and/or H3K4me2 in this analysis is unfortunate considering that these marks delineate distal cis-regulatory elements known to be more variable across cell types than promoter biased marks (H3K4me3 and H3K27me3).

2- ChromHMM analysis should be run on the data from all samples across all passages at once and then the proportion of peaks in one chromatin state as opposed to another should be reported across each passage. This would allow the reader to understand how different is the proportion of the genome in one chromatin state versus another and more readily report chromatin states that would be preferentially present in one passage as opposed to another..

3- The relation between changes in histone modification, DNA methylation and gene expression needs to be integrated to capture the relation between these different features. Figure 3G does not appear to effectively address this need.
4- More details in the methods are needed to properly assess the work. For example, linkage of DNA methylation changes to gene expression as discussed at the top of p5: using the 450K array annotation is not sufficient as CpG position with respect to the TSS determines the impact on gene expression. Changes in methylation and gene expression need to be paired based on the genomic feature in which the methylation change is measured (promoter, gene body etc…).
5- Relevance of model to mutant IDH tumor is lacking. Does the DNA methylation and expression profile of dox+ IHA correlate with the signal reported in primary tumor samples?
6- The premise of this work was to identify the mechanisms of partial response to mutant IDH-specific drugs. Are the "persistent" features identifying weaknesses to target in a complementary approach to mutant IDH-specific drugs?

Other major points:
7- The impact of mutant IDH1 expression on endogenous retroviral sequence expression is opposite to reports showing an increase in endogenous retroviral RNA production upon inhibition of DNA methylation/DNA demethylation (doi: 10.1016/j.cell.2015.07.056; doi: 10.1016/j.cell.2015.07.011). A more comprehensive characterization of chromatin methylation changes at all repetitive elements and a discussion on the relevance of the reported observation and past publications is warranted.
8- The relevance of mutant IDH1 expression on genomic instability should be investigated in greater depth. This is a novel and exciting aspect of the study, particularly as the impact on DNA methylation upon expression of mutant IDH in astrocytes has already been discussed by the authors in a previous study (Turcan

et al, Nature 2012; doi: 10.1038/nature10866). How many passages are required for the acquisition of CNA? How consistent are the CNAs acquired across biological replicates. Is there any particular chromatin methylation signature that relates to CNA regions? Etc,

9- The discussion establishes the relevance of discoveries made in this manuscript with only two past discoveries (1- 2HG inhibition is not sufficient to reverse mutant IDH induced chromatin changes and 2- the changes in the chromatin methylation was previously reported to impact expression of PDGFRA). This gives the impression that the current work has limited impact on the field.

Minor comments:

Figures

Fig1 b) and c): mutant IDH1 still present at dox off P10 (b, bottom panel), but no 2HG at P15 ? Please explain or show the IDH1 mutant detection at the same passage as the 2HG measurement.

Fig1 d): Re-arrange the labels

Fig2 e): show scale for each track

Fig3 b): Not adequate to support persisting methylation changes at 40P: could these not just be larger deltaBetas that have progressively shifted, in essence just more gradual changes instead of persisters? If these are indeed the same difference sin beta value at 40P, then this should be explicitly described in the legend and/or methods section.

Fig3 g): Re-arrange the display. Impossible to follow the methylation clusters with so many different colours and such small bars

Fig4 b): show scale for each track

Fig4 g): The figure does not match the text on p9 relating to Myc and ZNF143. A motif enrichment analysis would be more appropriate if the focus is on TF binding.

Main text
P7 par1: “...following dox withdrawal ; methylomes….” : sentence does not make sense
P9 par1:”We derived 11,443 most regions…”

Reviewer #2:
Remarks to the Author:
Recurrent mutations in IDH1 (and IDH2) were identified recently, relative to other oncogenes that occur with such high frequency. While these mutations present an obvious target for therapy, the epigenetic changes caused by IDH1 mutation do not appear to completely diminish upon chemical inhibition of the mutant enzyme. For this reason, the identification of the causative mutations in IDH1/2 has not yet led to an effective therapeutic strategy for deadly gliomas. The studies described here represent a deep inquiry into the longitudinal effects of the oncometabolite 2HG, and reveal interesting and potentially targetable characteristics of this unique epigenetic state.
The approaches used by the authors to determine the effects and duration of the 2HG responses are impressively comprehensive and quantitative. They begin with several independent cellular and tumorsphere models that are highly relevant to the glioma progenitors affected by mutations in IDH1. The inducible system they describe is particularly powerful, as it facilitates an elegant investigation into the temporal increases and decreases in DNA and histone methylation. Many novel and potentially useful findings are revealed. Among them is a mutant IDH1-dependent increase in the expression of the stem cell marker CD24, a reduction in the expression of the autophagy effector MAP1LC3A, and most interestingly an increase in genetic instability that appears to be related to the mobilization of endogenous retroviruses. To provide helpful context, the authors reference these dynamic changes to existing databases and atlases. The in

vivo/imaging studies provide further validation of the experimental system, and confirm the persistence of tumor growth despite shutting off IDH1 R132H expression. Thus, these tumors must not be “addicted” to 2HG, which is itself a critical, if not altogether unexpected, observation. What emerges from these studies is a unifying view of the central processes of tumorigenesis in astrocytes that will hopefully provide new strategies for treatment.
The paper is concise, well-written and appropriately referenced. The data figures are clearly presented and reasonably interpreted. In general, all central observations are robust and internally consistent. Several interesting observations remain somewhat thematically underdeveloped (e.g. the link to autophagy, mechanism of genetic instability, etc.). However, this is a very minor criticism given the already broad scope of this paper. The many singular observations that are reported here will doubtless be subjected to follow up in the years ahead.
Minor edit: the acronym CIMP should be defined at first use (p. 4)

Reviewer #3:
Remarks to the Author:
In this interesting manuscript, authors Turcan et al. show using inducible overexpression of IDH1R132H in immortalized astrocytes that IDH1 mutation mediated chromatin and DNA methylation changes can undergo some degree of reversibility upon the loss of IDH1R132H expression although this phenotype seems to vary according to locus for the time period tested. The authors have utilized a large suite of technologies to describe this reversibility including ChIP-Seq, DNA methylation arrays, RNA-Seq, and gene expression microarrays and have generated an impressive amount of novel data. Additionally, the highlighting of enrichment of activing histone marks dependent on IDH1R132H expression and call of attention to this often ignored phenotypic

consequence of IDH1 mutations is interesting. Unfortunately, there are several major weaknesses in the manuscript that significantly detract from its impact and generalizability, and several minor weaknesses in the clarity of data presentation.

Major Weaknesses:
1) Lack of clear connection to phenotypes in the human tumors, except in the case of CD24 expression which is found in the supplemental data. Do all of the genes in figure 1g show differences between CIMP+ and CIMP- LGG? How do you validate using Astrocytes as a model for LGG without doing comparisons?
2) Missing control, in figure 1h I would like to see the data for passage matched +dox cells or and –dox cells for each of the off dox passage data points to ensure that the CD24 expression was truly decreased due to the dox withdrawal. Another recurring problem throughout the manuscript is a lack of clarity about whether data points are being compared to passage matched controls or a baseline control in the figures.
3) Although figure 1i shows correlation between CD24 positivity and colony forming ability in passage 50 IHAs, whether IDH1-R132H correlated CD24 increasing expression actually contributes to the increase in colony forming ability in early IHAs is not directly assessed. Therefore, putting those two figures next to one another may mislead some readers as to the degree to which the data supports a functional role for CD24 in increasing clonogenic ability of IHAs.
4) No assessment of whether ERV expression leads to functional retrotransposition and therefore, genome instability. Perhaps the elements being transcribed and detected in the RNA-seq do not produce a functional retrotransposition event? Therefore, it may not be that “IDH1 R132H plays a role in de-regulating ERV retrotransposition which may contribute to genome instability or immune activation.” Also, was FPKM compared across samples in figure 5c? FPKM alone should not be compared due to differences

in effective library length and it requires additional normalizations. Are they significantly differentially expressed based on DESeq output, or did I just miss this statement somewhere?
5) Has a neuropathologist examined the murine lesions and diagnosed them as tumors? Or alternatively by what criteria are the murine lesions being called tumors?
6) In supplementary figure 5c there appears to be a potential excel gene name correction error, i.e. 1-Mar. Are the authors sure this has not occurred elsewhere in their dataset and skewed any results? The authors apparently did several gene enrichment analyses where this type of error could affect the outcome.
7) In figure 5e, the glioma cell line samples you've chosen do not recapitulate the expression differences found in the TCGA data for those loci in CIMP+ vs. CIMP- tumors, or for the grouping defined by IDH1 mutation for most of the genes observed. If a TCGA style quantitation of the H3K4me3 results was performed would the results derived from the GREAT toolbox actually reflect the human tumor condition accurately?
8) Due to the extensive utilization of exploratory statistical methods the authors should be more candid about which studies and samples they are drawing confirmatory conclusions from versus doing an initial exploratory data analysis.
Minor Weaknesses and Notes:
1) Several graphs found throughout the manuscript do not contain labeled axes or contain axes that are difficult to interpret based on the text description. i.e. Figure 1h is missing a labeled, and figure 3e seems to denote percent total but with the log scale it's difficult to evaluate how the low, medium, and high marks fall across the categories transient, gradual, and persistent. Figure 2G chromatin states could use further explanation of this approach.
2) I'd like a point of clarification as to whether the low cluster for the gene expression microarray data is also included in the medium cluster. The methods section says low is <1.5, medium <2. But is medium >1.5 and <2 or does it also contain the low grouping?
3) Tumor cells lines do not appear to be authenticated based on the

statement of utilization of only morphology and astrocytic expression markers. TS603 is an oligodendroglioma line according to previous publications from these authors.