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# Letters to the editor

## Comparison of immunohistochemistry and DNA sequencing for the detection of IDH1 mutations in gliomas

We read with interest a recent paper published by Chen et al on building a multivariable model to predict the likelihood of an IDH1/2 mutation in diffuse gliomas.[1] Incorporating patient age, glioblastoma diagnosis, and prior history of grade II or III gliomas, the model was shown to have high sensitivity and specificity for predicting the presence of an IDH1/2 mutation, either with or without an immunostain, and high accuracy for predicting the presence of a less common IDH1 or IDH2 mutation when the immunostain was negative. The authors suggested that the model will help triage diffuse gliomas that would benefit from mutation testing in both clinical and research settings.

We commend the authors for their effort to create such a model since IDH1/2 mutation has been shown to have both diagnostic and prognostic implications in diffuse gliomas. IDH1 in adult patients is associated with younger age at diagnosis, TP53 mutation, combined 1p/19q deletion, MGMT promoter hypermethylation, and favorable patient survival.[2] Detection of an IDH mutation has also been shown to be reliable for differentiating glioma from reactive gliosis.[2] There are even proposals to include IDH mutation status in the next version of the WHO classification of gliomas (Gupta 2011).[3] However, the utility of such a model to predict IDH mutation status in both clinical and research settings must be interpreted in the context of sensitivity and specificity of the 2 most common methods currently being used in the laboratory to detect this mutation: immunohistochemistry (IHC) and Sanger sequencing.

We identified 8 studies in the literature that directly compared IHC and sequencing in their detection of IDH1/2 mutation in gliomas (Table 1). The number of samples ranged from 49 to 343. Six studies included gliomas of all grades,[4–9] while 2 studies focused only on oligodendrogliomas.[10,11] The antibody used for IHC was DIA-H09 in 6 studies[4,5,8–11] and Imab in one study;[7] another study used both antibodies.[6] The concordance rate between IHC and sequencing ranged from 88% to 99%. In 5 of 8 studies, the number of mutations detected by IHC was greater than those detected by sequencing.[4,6–8,10] This was explained by the fact that only IHC can detect the mutation if there is only a small population of IDH1-R132H mutation-possessing tumor cells in the sample. Under model B proposed by Chen et al the predicted probability of IDH1 is 100% if IHC is positive. This makes the implicit assumption that as long as there are a few cells in the sample that stain positive for IDH-1 on IHC, the sample should be considered IDH1 positive. However, there is no study in the literature showing that glioma samples with only a small population of IDH1-R132H mutation-possessing tumor cells exhibit the same properties as those that are unequivocally IDH1 positive. In the remaining 3 studies, in which the number of mutations detected by sequencing was greater than those detected by IHC,[5,9,11] the most frequently cited reason for false negatives was that IHC had failed to detect the other types of IDH1 mutations including R132C (4%), R132L (1%), R132S (2%), R132G (2%), and IDH2 mutations.[2] To some extent in cases where immunostain is negative, the model proposed by Chen et al will generate the possibility of harboring a less common mutation

At our institution, we first test all diffuse glioma samples with IHC and only sequence the negative samples. If the model given by Chen et al predicts only a 20% likelihood of an IDH1 mutation in a negative IHC sample, will that change our decision to sequence the sample? The answer will be "no" if we believe that accurate assessment of the IDH1 status will factor significantly into how we prognosticate and manage the patient. The answer will only be "yes" if we are so resource

**Table 1.** Comparison of immunohistochemistry with sequencing for IDH1 testing in gliomas

| Study | Number of Samples | Tumor Type | Antibody Used | IDH1 Positive by ICH | IDH1 Positive by Sequencing | Concordance Rate |
|---|---|---|---|---|---|---|
| Capper 2009 | 186 | diffuse glioma | DIA-H09 | 102/186 | 101/186 | 92% (171/186) |
| Mellai 2011 | 343 | diffuse glioma | DIA-H09 | 60/270 | 63/270 | 99% (267/270) |
| Preusser 2011 | 95 | diffuse glioma | DIA-H09 | 66/95 | 65/95 | 92% (87/95) |
| Preusser 2011 | 95 | diffuse glioma | Imab-1 | 67/95 | 65/95 | 91% (86/95) |
| Takano 2011 | 49 | diffuse glioma | Imab-1 | 12/49 | 10/49 | 92% (45/49) |
| Lee 2012 | 141 | oligo | DIA-H09 | 107/141 | 105/141 | 94% (132/141) |
| Loussouarn 2012 | 91 | oligo | DIA-H09 | 47/90 | 55/90 | 91% (82/90) |
| Agarwal 2013 | 50 | diffuse glioma | DIA-H09 | 30/50 | 28/50 | 88% (44/50) |
| Catteau 2014 | 133 | diffuse glioma | DIA-H09 | 61/133 | 66/133 | 93% (124/133) |

constrained that the cost of sequencing outweighs the benefit provided by the information gained from accurate IDH mutation testing. Similarly, in a research setting, it is highly unlikely that any researcher would base the decision to immunostain or sequence the sample on what the model predicts.

In conclusion, we suggest that the models proposed by Chen et al demonstrating the clinical and pathological factors, which can be important for predicting IDH1/2 mutation in diffuse gliomas, currently have limited utility in both the clinical and research settings.

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# Reply to Letter

## Predicting the likelihood of an isocitrate dehydrogenase 1 or 2 mutation in diagnoses of infiltrative glioma

We greatly appreciate the interest and feedback provided by Zou et al regarding our recent paper describing a statistical model that predicts the probability of IDH1/2 mutations in adult gliomas.

One concern raised by Zou et al was that detection of a mutation via either immunohistochemistry and/or sequencing depends on the sensitivity and specificity of each method. In particular, specimens in which only a few scattered cells are R132H IDH1 immunopositive may not have the same properties as those with many more positive cells. Assuming the immunohistochemistry protocol is optimized, there are 2 reasons for only scattered cells showing R132H IDH1 immunopositivity: (i) the surgical biopsy did not capture fully diagnostic material, and (ii)the IDH1/2 mutant glioma was in the process of losing mutant protein expression. At our institutions (University of Kentucky and University of Pittsburgh), the Dianova R132H IDH1 antibody underwent validation prior to routine clinical implementation. Antibody specificity relative to sequencing was 100%, consistent with published data by the original developers of the antibody.[1,2] However, those cohorts only used tissue blocks with sufficient tumor cells to make a reliable WHO-based diagnosis. Likewise, the current study only used cases with unequivocal glioma. Indeed, that is why we said in the Discussion, "This application is also not meant for cases in which the biopsy