

## MASH1 expression in high grade astrocytoma may demonstrate a potential model for prognosis that may lead to a molecular target for future therapy

David Tacha, PhD., Jillian Tyrrell, PhD., and Margaret Lobo, MS.; Biocare Medical, Concord, CA

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### Introduction

Grade 1 astrocytoma can often be completely removed with surgery, however, in some patients the tumor can grow back.<sup>1</sup> In grade 2 astrocytoma (usually cannot be surgical removed), approximately 40% of people diagnosed with a grade 2 astrocytoma survive for 10 years or more after diagnosis. Studies have also shown that only 15 to 25% of patients diagnosed with grade 3 astrocytoma survived for 5 years or more after diagnosis.<sup>2,3</sup> Unfortunately, the prognosis is even worse for patients diagnosed with glioblastoma (grade 4), as most patients live for less than a year and about 2% of patients survive only three years.<sup>4</sup> The overall prognosis for glioblastoma has not changed much since the 1980s, despite major improvements in neurosurgery, radiotherapy and chemotherapy techniques.<sup>1</sup>

Achaete-scute complex homolog 1 (ASCL1 or MASH1) is a basic helix-loop-helix transcription factor essential for neuronal differentiation and specification in the nervous system and is present in proliferating progenitor cells.<sup>5</sup> In a recent study, the upregulation of MASH1 and inhibition of the notch signaling pathway characterize progressive astrocytoma.<sup>6</sup> They showed that the overall survival rates in grade 2 vs. grade 3 astrocytoma was sharply divided and morphological examination could not always distinguish between the two. The study used a polyclonal MASH1 antibody and only 37 cases of astrocytoma were evaluated.

Recently, a new commercially available MASH1 mouse monoclonal antibody has been shown to be a highly sensitive and specific marker for high-grade neuroendocrine carcinoma in various sites.<sup>7</sup> This led us to examine MASH1 expression in astrocytoma as a potential model for prognosis. In this study, we will evaluate the immunohistochemical expression of MASH1 in a series of grade 1 through grade 4 astrocytoma cases.

### Materials and Methods

Formalin-fixed paraffin-embedded tissue microarrays (TMA) consisting of samples of normal brain and astrocytoma previously diagnosed consisting of grade 1 (n=22), grade 2 (n=34), grade 3 (n=16) and grade 4 (n=35) were evaluated by immunohistochemical analysis using a monoclonal MASH1 antibody (Biocare Medical, Concord, CA). MASH1 (Class 1 IVD) was tittered at 1:100 and applied whole sections of normal brain and on TMA tissues for 60 minutes at room temperature. An HRP-polymer detection system was applied for 30 minutes, followed by DAB and lightly stained with hematoxylin. A value of >1% staining was scored as positive.

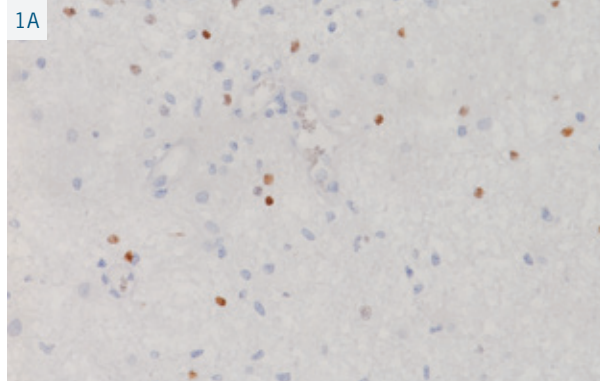
## Results

Results are summarized in Table 1. The overall staining of MASH1 was expressed in 61.7% (66/107) astrocytoma. In grade 1 astrocytoma, MASH1 was expressed in 31.8% vs. 69.4% in grades 2-4 ( $p \sim 0.0028$ ) (Figure 1A-D). However, grade 1 rarely expressed more than 5% (data not shown). In grade 2 astrocytoma, MASH1 expression was dramatically increased compared to grade 1 astrocytoma (85.3% vs. 31.8%), respectively. What was noted in grade 1 vs. grade 2 astrocytoma was a higher percentage of MASH1 stained cells in grade 2 vs. grade 1 (Figure 1A, B). In the majority of grade 1 astrocytoma, MASH1 expression was approximately 1 to 2% of total cells stained (data not shown). In grade 3 and 4 astrocytoma, 30/51 (58.9%), MASH1 expression was observed in a higher percentage of tumor cells (Figure 1C, D). In normal brain (cerebrum), MASH1 was expressed in a very low percentage of neurons (Figure 2).

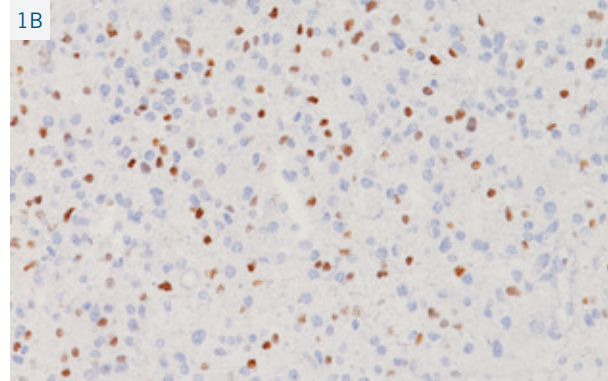
Table 1

Astrocytoma	(+) cases	(+) %
Grade 1	7/22	31.8%
Grade 2	29/34	85.3%
Grade 3	11/16	68.7%
Grade 4	19/35	54.3%

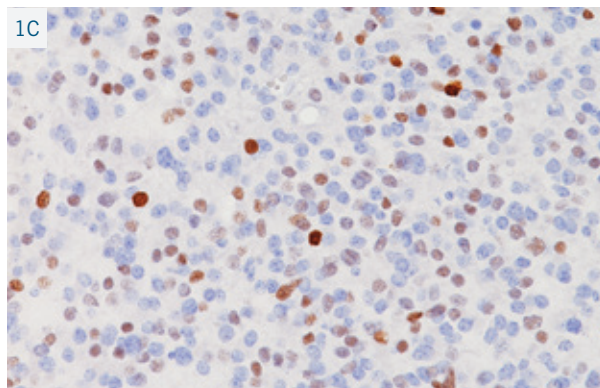
Figure 1



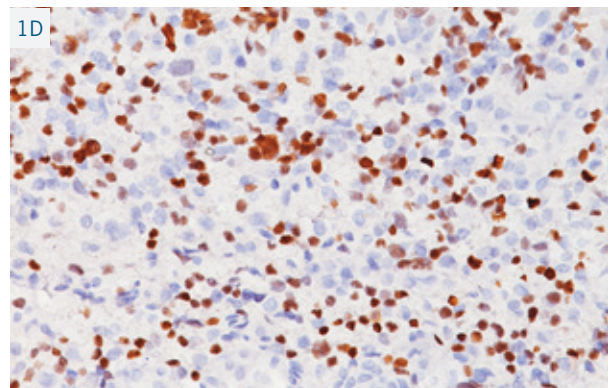
MASH1 expression in grade 1 astrocytoma



MASH1 expression in grade 2 astrocytoma

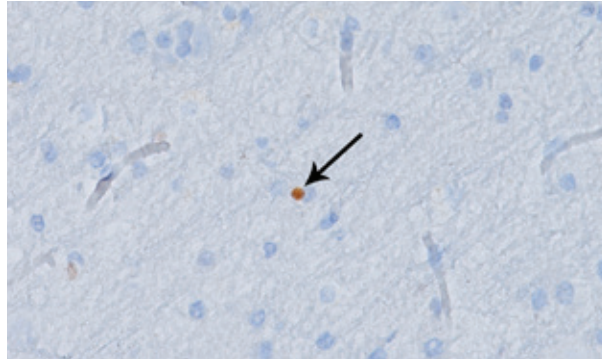


MASH1 expression in grade 3 astrocytoma



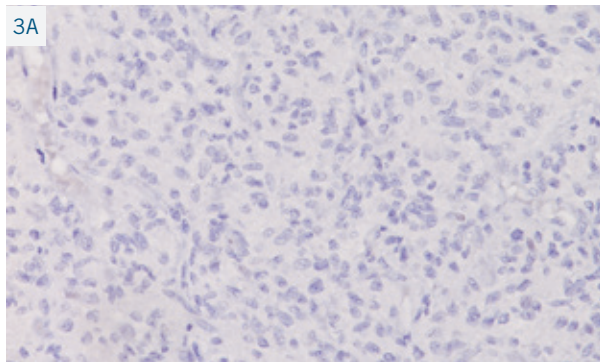
MASH1 expression in grade 4 astrocytoma (glioblastoma)

Figure 2

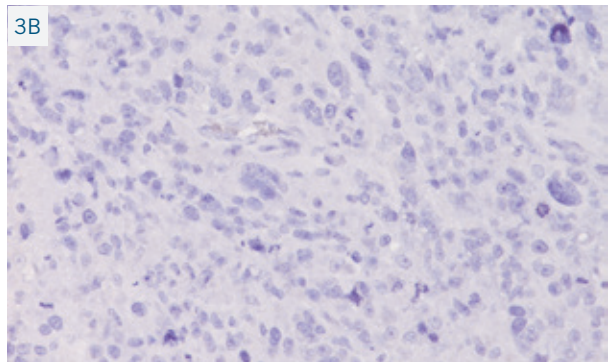


MASH1 expression in normal brain (arrow)

Figure 3



MASH1 (-) in grade 3 astrocytoma



MASH1 (-) in grade 4 astrocytoma

## Discussion

Only limited studies have compared MASH 1 expression in grades 1-4 astrocytoma.<sup>6</sup> This study represents the first study to use a mouse monoclonal MASH1 in grade 1-4 astrocytoma. Our results (Table 1) clearly shows a significant increase of MASH1 expression in grade 1 (31.8%) vs. in grade 2 (85.3%) astrocytoma. In grade 3-4 astrocytoma, 30/51 (59%) were positive and 20/51 (39%) were negative (Figure 3 A, B); and thus, levels of MASH1 expression is found in the majority of grade 3-4 astrocytoma. Somasundaram, *et al*, also demonstrated the upregulation of MASH1 was progressive in astrocytoma.<sup>6</sup> The high expression pattern of MASH1 may represent a biological and pathophysiological difference in tumor aggression.

In a study by Hiroshima *et al*, patients with high MASH1 expression had a poorer 5-year overall survival (48/100%) in small cell lung carcinoma.<sup>8</sup> Neuroendocrine differentiation in prostate cancer is a well-recognized phenotypic change by which prostate cancer cells transdifferentiate into neuroendocrine-like tumor cells; and neuroendocrine differentiation in prostatic adenocarcinomas has been associated with poor prognosis.<sup>9,10</sup> Shida, *et al* also demonstrated high MASH1 expression in gastroenteropancreatic neuroendocrine tumors in which patients were associated with a significant shorter overall survival rate.<sup>11</sup> Therefore, high MASH1 and with other associated gene expressions is not only a potential prognostic marker, but the identification of MASH1 positive cancers could be a candidate for new targets therapeutic strategies. New advances in the treatment of aggressive neuroendocrine lung cancers are needed to improve survival in patients with these types of tumors. ASH1 gene has been shown to be a specific therapeutic target for lung cancers with neuroendocrine features.<sup>12</sup> In a study by Osada *et al*, the authors identified that the transcriptome of ASCL1 is both necessary for the proper development of neuroendocrine cells and essential for the growth and survival of neuroendocrine lung cancers.<sup>13</sup> Hopefully, these kinds therapies for lung cancer can be adopted in the future for patients with astrocytoma.

## Conclusion

MASH1 may be a potential mechanism in the aggressive behavior in high-grade astrocytoma. Our results showing the variable and progressive expression of MASH1 in high-grade astrocytoma may demonstrate not only a potential model for prognosis, but could lead to future target therapeutic strategies.

## References

1. [www.cancerresearchuk.org](http://www.cancerresearchuk.org)
2. Sarica FB, *et al.* Five-year follow-up results for patients diagnosed with anaplastic astrocytoma and effectiveness of concomitant therapy with temozolomide for recurrent anaplastic astrocytoma. *Asian J Neurosurg.* 2012 Oct; 7(4):181-90.
3. Cabanas R, *et al.* Treatment of children with high grade glioma with nimotuzumab: a 5-year institutional experience. *MAbs.* 2013 Mar-Apr; 5(2):202-7.
4. Walid MS. Prognostic factors for long-term survival after glioblastoma. *Perm J.* 2008 Fall;12(4):45-8.
5. Kim EJ, *et al.* Ascl1 (Mash1) lineage cells contribute to discrete cell populations in CNS architecture. *Mol Cell Neurosci.* 2008 Aug;38(4):595-606.
6. Somasundaram K, *et al.* Upregulation of ASCL1 and inhibition of Notch signaling pathway characterize progressive astrocytoma. *Oncogene.* 2005 Oct 27;24(47):7073-83.
7. La Rosa S, *et al.* Achaete-scute homolog 1 as a marker of poorly differentiated neuroendocrine carcinomas of different sites: a validation study using immunohistochemistry and quantitative real-time polymerase chain reaction on 335 cases. *Hum Pathol.* 2013 Jul;44(7):1391-9.
8. Hiroshima K, *et al.* Distinction of pulmonary large cell neuroendocrine carcinoma from small cell lung carcinoma: a morphological, immunohistochemical, and molecular analysis. *Mod Pathol.* 2006 Oct;19(10):1358-68.
9. Hu CD, *et al.* Neuroendocrine differentiation in prostate cancer: a mechanism of radioresistance and treatment failure. *Front Oncol.* 2015 Apr 14;5:9.
10. Gupta A, *et al.* Mash1 expression is induced in neuroendocrine prostate cancer upon the loss of Foxa2Prostate. 2013 May;73(6):582-9.
11. Shida T, *et al.* The expression of NeuroD and mASH1 in the gastroenteropancreatic neuroendocrine tumors. *Mod Pathol.* 2008 Nov;21(11):1363-70.
12. Augustyn A, *et al.* ASCL1 is a lineage oncogene providing therapeutic targets for high-grade neuroendocrine lung cancers. *Proc Natl Acad Sci U S A.* 2014 Oct 14;111(41):14788-93.
13. Osada H, *et al.* ASH1 gene is a specific therapeutic target for lung cancers with neuroendocrine features. *Cancer Res.* 2005 Dec 1;65(23):10680-5.