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Diagnostic and prognostic value of alpha internexin expression in a series of 409 gliomas

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ABSTRACT

The neuronal intermediate filament alpha internexin (INA) is expressed in most gliomas with 1p19q codeletion and could represent a valuable prognostic marker in clinical routine. INA expression was analysed on 409 gliomas and correlated with histology, progression free survival (PFS), overall survival (OS), genomic profile assessed by CGH-array, *IDH1/IDH2* mutation and p53 expression. INA was expressed in 59% of grade II oligodendrogliomas ($n = 73$), 45% of grade III oligodendrogliomas ($n = 133$), 15% of grade II oligoastrocytomas ($n = 61$), 12% of grade III oligoastrocytomas ($n = 41$), 23% of glioblastomas with oligodendroglial component ($n = 31$), 0% of grade I astrocytomas ($n = 3$), 0% of grade II astrocytomas ($n = 14$), 6% of grade III astrocytomas ($n = 17$) and 0% of glioblastomas ($n = 36$). INA expression was detected in 85% of gliomas with complete 1p19q codeletion ('true 1p19q signature') ($n = 85$) versus 15% of gliomas without 1p19q codeletion ($n = 245$), including 14% of gliomas with variable/partial 1p19q deletion ('false 1p19q signature') ($n = 72$) ($p < 0.0001$). INA was expressed by 43% of gliomas with *IDH1* mutation ($n = 197$) versus 12% of gliomas without *IDH1* mutation ($n = 156$) ($p < 0.0001$). In oligodendroglial gliomas ($n = 240$), INA expression specificity for 1p19q codeletion was 80%, sensitivity 85%, positive predictive value 70%, and negative predictive value was 91%. Combining INA and p53 expressions improved INA predictive accuracy for 1p19q codeletion. In grade III gliomas, INA expression was associated with longer PFS (42.1 versus 10.2 months, $p = 0.0007$) and longer OS (124.6 versus 20.6 months, $p = 0.0001$). In conclusion, INA expression is a fast, cheap and reliable prognostic marker, and represents a surrogate marker for 1p19q complete codeletion.

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1. Introduction

Current histological classification of gliomas is still based on subjective criteria – both for phenotype determination and grading. It lacks reproducibility and precision in terms of prognosis, particularly for grade II/III gliomas. Genetic subtyping of a given histological phenotype and robust biomarkers has improved the prognostic assessment: the chromosome arms 1p/19q codeletion characterises a subtype of oligodendroglial tumours with better prognosis and higher chemosensitivity.[1,2] More recently, IDH1 (more rarely IDH2) mutations have been found in nearly 40% of gliomas and strongly predict better outcomes.[3–7] Using gene expression arrays we have shown that 1p19q codeleted gliomas (mostly oligodendrogliomas) express preferentially neural genes. We found that alpha internexin (INA) was one of the most differentially expressed genes between 1p19q codeleted and *EGFR* amplified gliomas.[8] INA is a proneural gene located on 10q24.33, encoding a neurofilament interacting protein. A retrospective study on 112 gliomas has shown that INA expression was tightly related to 1p19q codeletion and was also predictive of good outcome in anaplastic oligodendrogliomas (OIII) and anaplastic oligoastrocytomas (OAIII).[9] The aim of the present study was to assess the diagnostic and prognostic value of INA expression in a series of 409 adult gliomas.

Table 1 – INA expression, 1p19q loss, *IDH1* mutation and p53 expression according to histology.

	INA expression	1p19q loss	*EGFR* amp.	*IDH1* mutation	p53 expression
Pilocytic astrocytoma ($n = 3$)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	33% (1/3)
Grade 2 astrocytoma ($n = 14$)	0% (0/14)	0% (0/12)	8% (1/12)	46% (6/13)	71% (10/14)
Grade 3 astrocytoma ($n = 17$)	6% (1/17)	0% (0/15)	20% (3/15)	53% (8/15)	76% (13/17)
Glioblastoma ($n = 36$)	0% (0/36)	0% (0/35)	40% (13/32)	20% (7/34)	60% (21/35)
Glioblastoma with oligodendroglial component ($n = 31$)	22% (7/31)	0% (0/27)	15% (4/27)	41% (12/29)	88% (23/26)
Grade 2 oligodendroglioma ($n = 73$)	59% (43/73)	49% (29/59)	0% (0/59)	69% (40/58)	28% (19/67)
Grade 3 oligodendroglioma ($n = 133$)	45% (60/133)	55% (51/93)	12% (11/93)	53% (61/115)	37% (32/86)
Grade 2 oligoastrocytoma ($n = 61$)	15% (9/61)	6% (3/53)	2% (1/53)	79% (39/49)	60% (36/60)
Grade 3 oligoastrocytoma ($n = 41$)	12% (5/41)	6% (2/35)	14% (5/35)	63% (24/38)	71% (23/38)
Total	409	329	329	353	346

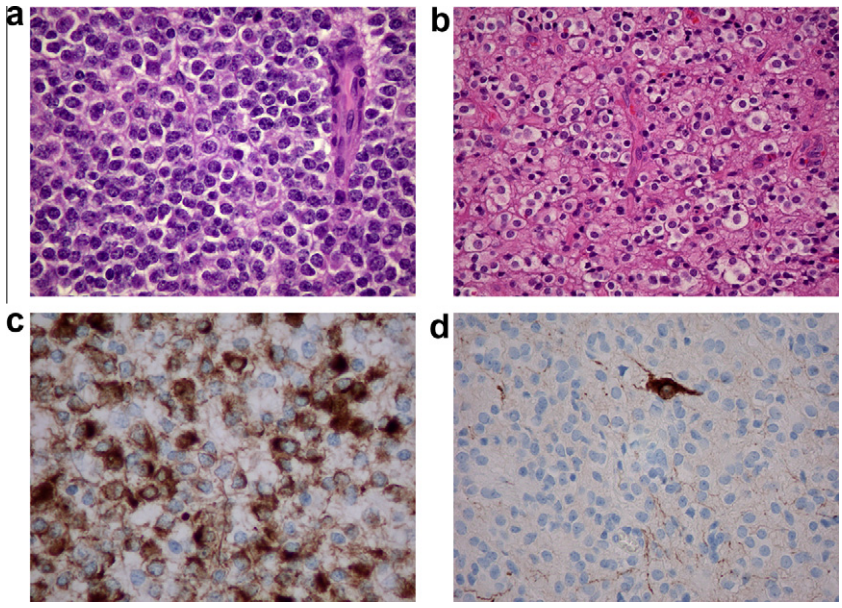

Fig. 1 – Two examples of anaplastic oligodendrogliomas: histopathological findings (HE) (a,b); INA expression analysis (immunohistochemistry) (c,d); genomic profile (e,f) analysed with high density SNP beadchip (ref HumanCytoSNP-12, Illumina). Anaplastic oligodendroglioma with 'true 1p19q signature' (a,c,e) – a: typical honeycomb pattern with microvascular proliferation – c: diffuse intracytoplasmic expression of INA – e: the genomic profile shows the loss of whole 1p (top) and whole 19q chromosome arm (bottom) with a centromeric breakpoint (arrows). Anaplastic oligodendroglioma with 'false 1p19q signature' (b,d,f): b: Oligodendroglial proliferation with vascular hyperplasia and high mitotic activity (not shown) – d: absence of expression of internexin alpha with a residual neuron labelled by the antibody. – f: the genomic profile shows the partial loss of 1p (top; the arrow showing the breakpoint) and the complete loss of both19p and 19q chromosomes arms (bottom).

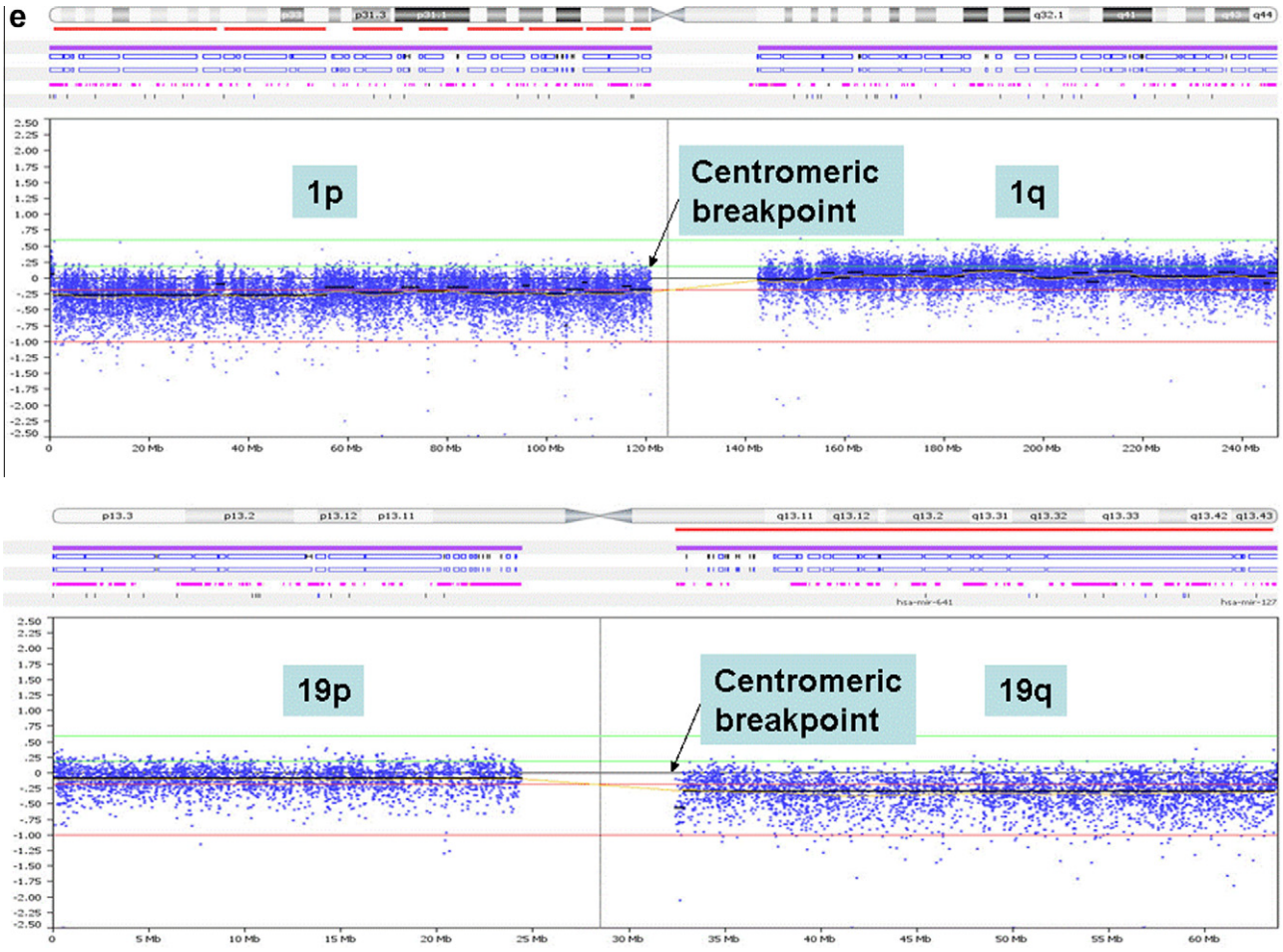

Fig 1. *(continued)*

2. Materials and methods

INA immunohistochemistry was performed on histological sections of formalin-fixed paraffin-embedded tumor samples in a series of 409 adult patients with gliomas, which included the previously published 112 cases.[9] The clinical annotations were retrieved from the database of the Pitié-Salpêtrière neurooncology department. Patients provided written informed consent for this study.

INA immunohistochemistry was carried out on 3 μm thick paraffin embedded sections of formalin-fixed tumor samples using an antibody directed against the neuronal intermediate filament internexin alpha (INA) (Novus Biologicals, Interchim, diluted 1/100) as previously described.[9] The immunolabelling technique was performed by a BenMark XT automated tissue staining systems (Ventana Medical Systems Inc.). Only INA positive intracytoplasmic crescents or paranuclear dots were considered. The percentages of INA positive cells were evaluated semi-quantitatively as follows: <10%, 10–24%; 25–50%, >50%. INA immunohistochemistry was considered positive only when more than 10% of the cells were positive. In positive cases, the labelling could involve most of the tumor cells ('diffuse' labelling), cluster of cells ('clustered') or isolated cells ('sparse'). Isolated entrapped INA positive neurons or axons were not considered. Slides were read by KM according to the above criteria.

p53 immunohistochemistry, CGH-array analysis and *IDH1/IDH2* mutation assessment were performed as previously described.[10–12] Most of the 1p19q codeletions were detected with BAC-array[11]; other were analysed with high density SNP beadchip (ref HumanCytoSNP-12, Illumina) that features approximately 300,000 genetic markers. These techniques identified complete 1p/19q codeletion-referred here as 'true 1p19q signature' – from variable 1p (typically 1p36) and 19q partial deletions, – referred here as 'false 1p19q signature' or '1p19q like'.

Histology, 1p19q codeletion status assessed by CGH-array, *IDH1* mutation status, *EGFR* amplification assessed by CGH-array, and p53 expression of INA positive and INA negative tumours were compared. Chi-square tests were used to compare the number of INA positive cases in the various tumour groups. Progression free survival (PFS) and overall survival (OS) in OIII and OAIII of cases with INA positive and INA negative tumours were compared. OS was defined as the time from diagnosis to death or last follow-up. PFS was defined as the time from diagnosis to recurrence or last follow-up. Patients lost for follow-up were censored on the last known day of life. Kaplan–Meier methods were used to obtain

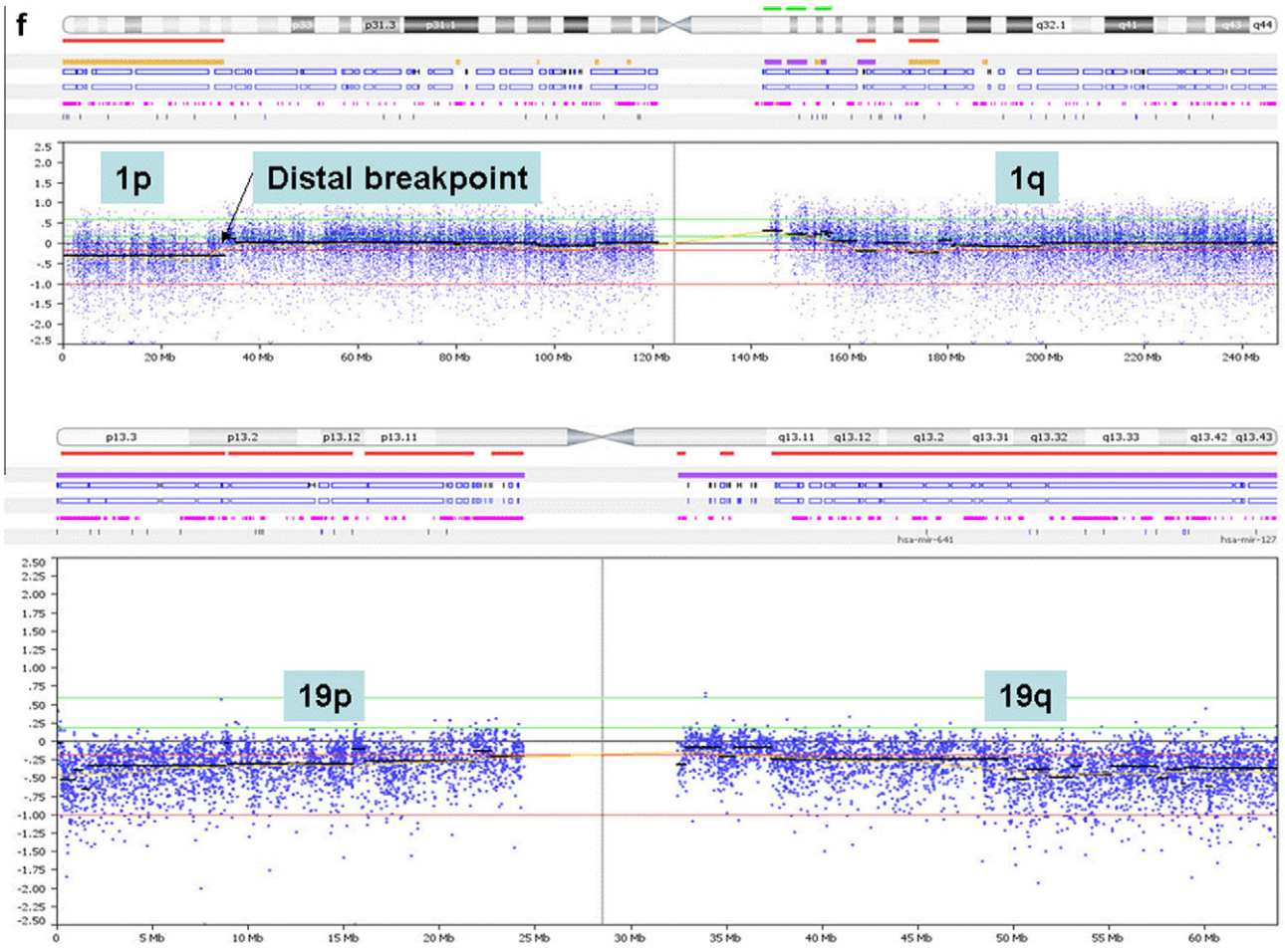

Fig 1. *(continued)*

survival curves, and a two-sided Log rank test was used to compare strata. A multivariate Cox regression model was used to adjust the effect of INA expression on OS or PFS for age, type of surgery, Karnofsky Performance Status (KPS), postoperative treatment, 1p19q status according to CGHa, *IDH1* mutation, *EGFR* amplification. Independent covariates were selected by stepwise methods.

3. Results

The histology, INA expression, 1p19q codeletion status, *IDH1* mutation status and p53 expression of the 409 gliomas are reported in Table 1. INA expression was detected in 59% of grade II oligodendrogliomas (OII), 45% of grade III oligodendrogliomas (OIII), 15% of grade II oligoastrocytomas (OAII), 12% of grade III oligoastrocytomas (OAIII), 23% of glioblastomas with an oligodendroglial component (GBMO), 0% of grade I astrocytomas (AI), 0% of grade II astrocytomas (AII), 6% of grade III astrocytomas (AIII) and 0% of glioblastomas (GBM).

IDH1 mutation analysis could be performed in all 409 samples, p53 immunohistochemistry in 346 samples and CGH-array analysis in 329 samples. CGH-array could distinguish clearly 'true 1p19q signature' from 'false 1p19q signature' (Figs. 1 and 2). INA expression according to histology (oligodendroglial versus astrocytic tumours), 1p19q codeletion, *IDH1* mutation, *EGFR* amplification and p53 expression are reported in Table 2. INA expression was tightly related to pure oligodendroglial phenotype ($p < 10^{-4}$), to 1p19q codeletion ($p < 10^{-4}$) and to *IDH1* mutation ($p < 10^{-4}$) whereas it was negatively correlated with *EGFR* amplification ($p = 0.002$) and p53 expression ($p < 10^{-4}$). In oligodendroglial tumours (OII, OIII, OAII and OAIII, $n = 240$), INA expression specificity for 1p19q codeletion was 80% (125/156), sensitivity 85% (72/85), positive predictive value 70% (72/103), and negative predictive value 92% (125/138). Combining INA and p53 expressions improved INA predictive accuracy for 1p19q codeletion: 53/68 cases with positive INA and negative p53 expression had a 1p19q codeletion (positive predictive value = 78%) versus only 2/131 cases with negative INA and positive p53 expression (negative predictive value = 98.5%). Seventy-two gliomas displayed a 'false' 1p19q codeletion (variable partial deletions or gains involving 1p and 19q) that could have been misinterpreted as a 'true' one if the LOH technique had been used. INA expression was negative in 86% (62/72) of these cases (Figs. 1 and 2). INA expression was inversely correlated with the loss of chromosome 10q (that contains le gene encoding INA on 10q24.33) ($p < 10^{-4}$). This may merely reflect the fact that 'true' 1p19q codeletion and loss of chromosome 10q are mutually exclusive ($p < 10^{-4}$). In fact, when the true 1p19q codeletion was excluded, there was no correlation between INA expression and 10q deletion (Fig. 2).

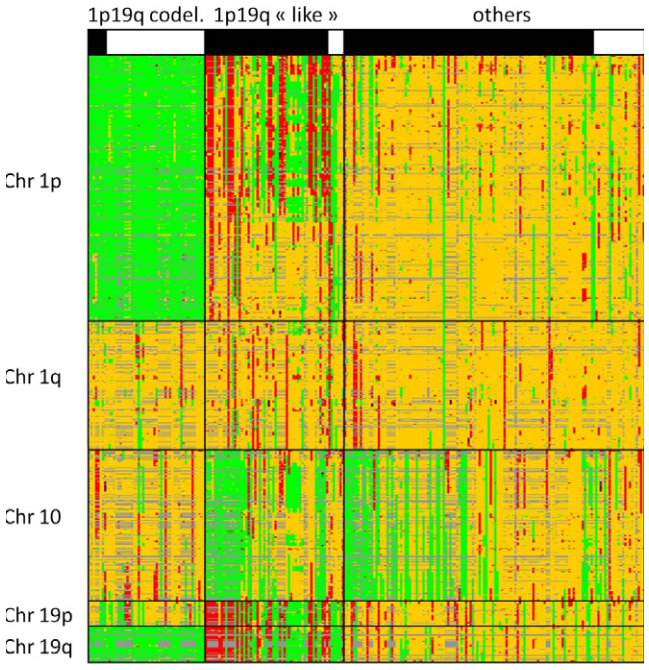

Fig. 2 – INA expression according to the genomic profile of the gliomas assessed by CGH array. Each column corresponds to a sample. Lines correspond to probes located on chromosomes 1, 10 and 19. Yellow colour indicates normal genomic copy number, green indicates a loss (see the loss of 1p and 19q) and red indicates a gain. The order of the samples, from left to right, is the following: gliomas with a true 1p19q codeletion (loss of the whole 1p and the whole 19q), gliomas with a false 1p19q codeletion or 1p19q 'like' (variable partial deletions or gains involving 1p and 19q that might be misinterpreted as a true 1p19q codeletion when using the LOH technique, particularly when a few probes are used). On the top of the figure, black indicates positive and white negative INA expression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2 – INA expression according to oligodendroglial phenotype, 1p19q loss, *IDH1* mutation, *EGFR* amplification and p53 expression; the *p* value refers to the Chi square test.

	Positive INA expression (% of samples)	
Pure oligodendroglioma		
Yes ($n = 206$)	103 (50%)	$p < 10^{-4}$
No ($n = 203$)	22 (11%)	
1p19q codeletion		
Yes ($n = 85$)	73 (85%)	$p < 10^{-4}$
No ($n = 244$)	38 (15%)	
IDH1 mutation		
Yes ($n = 197$)	85 (43%)	$p < 10^{-4}$
No ($n = 156$)	19 (12%)	
EGFR amplification		
Yes ($n = 38$)	2 (5%)	$p = 0.0002$
No ($n = 291$)	109 (37%)	
p53 expression		
Yes ($n = 182$)	29 (16%)	$p < 10^{-4}$
No ($n = 164$)	72 (43%)	

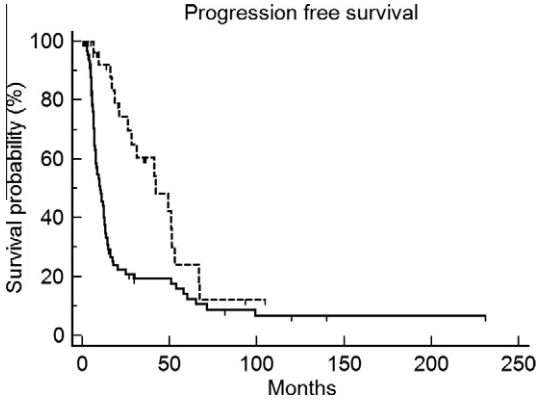

Fig. 3 – Progression free survival in anaplastic gliomas according to INA expression. Continuous line = INA–, broken line = INA+; $p = 0.0007$.

The impact of INA expression was evaluated on survival of grade 3 and grade 4 gliomas (follow-up was insufficient in grade 2 gliomas). INA expression was not associated with improved PFS or OS in grade 4 patients, but only 7 out of the 61 patients were positive. In grade 3 gliomas (OIII, OAIII, AIII, $n = 125$), INA expression was associated with longer PFS (42.1 versus 10.2 months, $p = 0.0007$) (Fig. 3) and longer OS (124.6 versus 20.6 months, $p = 0.0001$) (Fig. 4). Similar results were obtained when considering only anaplastic oligodendroglial tumours (OIII and OAIII) (PFS: 42.1 versus 9.5 months, $p = 0.0003$, OS: not reached versus 19.5 months, $p < 10^{-4}$). The 1p19q codeletion had also a strong favourable impact on survival: PFS was 42.1 versus 10.2 months ($p = 0.0006$) and OS was 124.6 versus 28 months ($p = 0.0001$), for deleted versus non-deleted tumours. In the subgroup of anaplastic gliomas with *IDH1* mutation ($n = 55$), INA expression was associated with longer OS (80.5 versus 46.2 months, $p = 0.04$) (Fig. 5), but not significantly with longer PFS (50.7 versus 14.2 months, $p = 0.3$). The number of patients with INA expression without

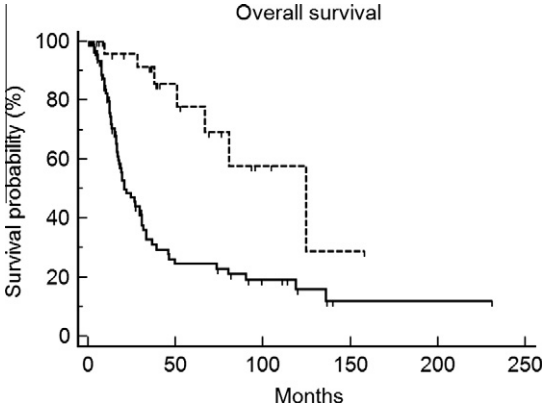

Fig. 4 – Overall survival in anaplastic gliomas according to INA expression. Continuous line = INA–, broken line = INA+; $p = 0.0001$.

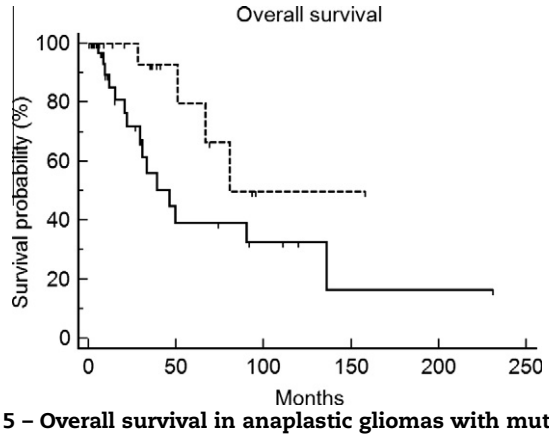

Fig. 5 – Overall survival in anaplastic gliomas with mutated *IDH1* according to INA expression. Continuous line = INA–, broken line = INA+; $p = 0.04$.

1p19q codeletion or without *IDH1* mutation was too small to assess INA prognostic values in these subgroups.

In addition to INA expression and 1p19q codeletion, age, Karnofsky performance status (KPS), type of surgery (biopsy versus tumour removal), *IDH1* mutation and *EGFR* amplification were all related to survival. Cox regression multivariate analysis indicated that the hazard ratio (HR) associated with INA expression, adjusted for age, KPS, type of surgery, *IDH1* mutation and *EGFR* amplification, remained significant (HR = 0.11. 95% confidence interval, 0.03–0.42; $p = 0.001$); however, when adjusted for 1p19q codeletion, HR associated with INA expression was no longer significant, confirming that both variables were tightly related. Similarly in the whole series, when taking into account the previous factors (except the 1p19q codeletion) and also grade and phenotype (oligodendroglial versus mixed and astrocytic), INA expression remained significant (HR = 0.36. 95% confidence interval, 0.16–0.82; $p = 0.01$).

4. Discussion

The 1p19q codeletion, the *MGMT* promoter methylation and the *IDH1* mutation are currently the most important prognostic biomarkers in adult gliomas. However, their assessment requires molecular biology techniques that in contrast to immunohistochemistry are not available worldwide and not always feasible. The present study of 409 gliomas confirms our preliminary results showing that INA expression is tightly related to oligodendroglial histology and to 1p19q codeletion.[9] The 1p19q codeletion is related to an unbalanced t(1;19)(q10;p10) translocation and represents the strongest prognostic factor in oligodendrogliomas.[2,13,14] Direct test for translocation is rarely performed in clinical practice: 1p19q codeleted oligodendrogliomas are difficult to grow. The caryotype cannot, therefore, be routinely obtained. The translocation can be suspected using a fluorescent in situ hybridization (FISH) fusion test with two probes flanking the breakpoint on the 1q19p derived chromosome.[13] However, in practice, the most widely available techniques detect the 1p19q codeletion and not the translocation. They include FISH, LOH, multiplex ligation-dependent probe amplification (MLPA) and CGH-array; the last is the most complete, reliable and sophisticated but also the most expensive technique. In fact, all of them have specific requirements and drawbacks: contamination with normal cells may impair the genetic analysis and the amount of available tissue, in case of biopsy, may be insufficient for CGH-array; LOH and FISH may be inadequate to distinguish the 'true' from the 'false 1p19q signature': CGH-array analysis (Figs. 1 and 2) shows that a substantial number among the 'false 1p19q signature' (73/329) could have been misdiagnosed as 1p19q codeleted by routine FISH or by LOH analysis. The use of a limited number of probes may not discriminate partial from complete deletion; for example distal 1p deletion is frequently associated with 19 chromosome loss (Fig. 1) and has a radically different prognosis.[15] In addition LOH imbalance on 1p19q may be due to chromosomal gain.

We further demonstrate that INA expression is overrepresented in tumours with *IDH1* mutation and underrepresented in tumours with p53 expression and *EGFR* amplification. INA expression can be assessed quickly from a simple biopsy, is reliable and inexpensive and does not need any special equipment. In our preliminary work, three types of INA expression patterns were distinguished: negative, weak (<10% cells) and strong (>10% cells). With more experience, we propose now to classify samples merely as positive (>10% cells) and negative (<10% cells). The present series suggests that INA may be used as a surrogate marker of 1p19q codeletion. The absence of INA expression in an oligodendroglial tumour makes the 1p19q codeletion very unlikely (11 out of 125 cases = 9%) particularly if the tumour is p53 positive (1 out of 71 cases = 2%). In contrast, a tumour expressing INA has a 70% chance to be 1p19q codeleted and an 80% chance if p53 is negative. Interestingly, INA expression was positive in 87% of tumours with a 'true' 1p19q codeletion, but only 14% of tumours with a 'false' 1p19q codeletion, suggesting that INA expression might help differentiating 'true' and 'false' 1p19q codeletions when 1p19q codeletion is assessed by the LOH technique. We also confirm that INA expression is associated with favourable outcome in anaplastic gliomas. Up to now, assessment of INA expression is the simplest tool to identify anaplastic gliomas with better prognosis. Antibodies directed against the mutated $IDH1^{R132H}$ protein are being developed and recent reports suggest that it is a promising tool for diagnostic and prognostic assessments in gliomas.[16–18] Our data show that the two assessments are not redundant but rather complementary: even in the *IDH1* mutated subgroup of grade III gliomas INA expression is associated with prolonged OS (80.5 versus 46.2 months, $p = 0.04$). This suggests that combining both INA and $IDH1^{R132H}$ expressions may be a simple test for prognostic stratification of anaplastic gliomas. This conclusion is in line with our previous data showing that grade II and grade III gliomas can be separated in three prognostic subgroups according to IDH1 and 1p19q genetic status.[19]

Conflict of interest statement

None declared.

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