รายงานวิจัยฉบับสมบูรณ์

การศึกษาความผิดปรกติทางพันธุกรรมและระดับโมเลกุลที่สามารถพยากรณ์การอยู่รอดและการตอบสนองต่อการรักษาโรคเนื้องอกสมองชนิด oligodendroglial tumors ในผู้ป่วยไทย

โดย

นายแพทย์สิทธิ์ สาธรสุเมธี

กุมภาพันธ์ พ.ศ. 2556

สัญญาเลขที่ MRG5280209

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และสำนักงานกองทุนสนับสนุนการวิจัย

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บทคัดย่อ

วัตถุประสงค์: เพื่อประเมินการขาดของโครโมโซม 1p และ 19q การกลายพันธุ์ของ isocitriate dehydrogenase (IDH)-1 ชนิด R132H การแสดงออกของ carbonic anhydrase (CA)-9 และ hypoxia-inducible factor (HIF)-2α ในเนื้องอกสมอง oligodendroglioma และประเมินความสัมพันธ์ระหว่างลักษณะทางพยาธิดังกล่าวด้วยกันเองและระหว่าง ลักษณะนี้กับอัตราอยู่รอดของผู้ป่วย

วัสดุและวิธีทดลอง: เนื้องอกสมอง oligodendroglioma จากผู้ป่วย 26 รายบน tissue microarray (TMA) ได้ถูกตรวจด้วย Fluorescence in situ hybridization (FISH) เพื่อหาการขาดของโครโมโซม และ immunohistochemistry เพื่อตรวจหา IDH1 R132H และการแสดงออกของ CA-9 และ HIF-2α

ผลการทดลอง: ความถี่ของการขาดของโครโมโซม 1p, 19q และทั้ง 1p/19q เป็นร้อยละ 65, 85 และ 62 ตามลำดับ การกลายพันธุ์ IDH1 R132H พบได้ร้อยละ 65 ภาวะการขาดร่วมของโครโมโซม 1p และ 19q สัมพันธ์กับอัตราการอยู่รอดสุทธิที่ยืนยาวและการตอบสนองต่อการฉายรังสีที่ดี การกลายพันธุ์ IDH1 R132H มีความสัมพันธ์กับการอยู่รอดที่ดีเช่นกัน การแสดงออกในระดับสูงของ CA-9 พบได้ร้อยละ 31 และสามารถบ่งชี้ภาวะการขาดร่วมของโครโมโซม 1p/19q ได้ร้อยละ 50 (P = 0.0095) ส่วน HIF-2α นั้นไม่มีความสัมพันธ์กับการพยากรณ์การอยู่รอดของผู้ป่วย

สรุปผลการทดลอง: CA-9 ซึ่งเป็นตัวบ่งชี้ของภาวะขาดอ็อกซิเจนและความเป็นกรดในเนื้องอก สามารถบ่งชี้การขาดร่วมของโครโมโซม 1p และ 19q ได้ในเนื้องอก oligodendroglioma บางกลุ่ม ซึ่งอาจทำให้ CA-9 เป็นการตรวจคัดกรองแบบง่ายเพื่อหาการขาดร่วมของโครโมโซมดังกล่าว และอาจเป็นเป้าหมายใหม่ของการรักษาเนื้องอก oligodendroglioma ที่มีการขาดร่วมของโครโมโซม

คำหลัก: oligodendroglioma, IDH, โครโมโซม 1p และ 19q, hypoxia

Abstract

Project Code: MRG5280209

Project Title: Genetic and molecular predictors of survival and therapeutic response in Thai patients with oligodendroglial tumors

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Project Period: 2009-2011 (Extended to 2013)

Abstract

Objectives: we aimed to evaluate the status of 1p and 19q, IDH1 R132H mutation and expression of hypoxic markers including carbonic anhydrase (CA)-9 and hypoxia-inducible factor (HIF)-2α in oligodendrogliomas. Correlations between these markers and associations between these markers and survival outcomes were conducted.

Material and methods: Twenty-six oligodendrogliomas were processed into tissue microarray (TMA). Fluorescence in situ hybridization (FISH) was exploited to detect chromosome deletion, whereas immunohistochemistry was performed to assess IDH R132H mutation, CA-9 and HIF-2α expression.

Results: The frequencies of isolated 1p deletion, isolated 19q deletion and co-deletion of 1p/19q were 65%, 85% and 62% respectively. IDH1 R132H mutation was detected in 65%. Co-deletion of 1p and 19q was associated with longer progression-free survival (PFS) after radiotherapy and improved overall survival (OS). Similarly, IDH1 R132H mutation was associated with better survival outcomes. High expression of CA-9 was observed in 31% and it identified 50% of oligodendroglioma with co-deletion of 1p/19q ($p = 0.0095$). However, another hypoxic marker, hypoxia-inducible factor-2α provided no additional prognostic value for survival.

Conclusions: CA-9, a marker for hypoxia/acidosis, identifies a subset of oligodenrogliomas with co-deletion of 1p/19q with favorable prognosis. CA-9 may serve as a simple screening test for the co-deletion and represent a new therapeutic target for co-deleted oligodendroglioma.

Keywords: oligodendroglioma, IDH, 1p, 19q, hypoxia

Background and Significance

Oligodendrogliomas

Primary brain tumors account for less than 2% of all cancers in adult, however they are often associated with neurologic morbidity and high mortality.[1,2] Gliomas are the most common primary brain tumors and can be classified based on histology into four types; astrocytomas, oligodendrogliomas, mixed oligoastrocytomas and ependymomas.[3] Pure oligodendrogliomas have a better prognosis than astrocytic tumors of the same grade, whereas the prognosis of mixed oligoastrocytomas is in between these two histopathologies. Oligodendroglial tumors (oligodendrogliomas and mixed oligoastrocytomas) are distinguished from other subtypes by their remarkable sensitivity to chemotherapy and long-lasting response to radiotherapy.[4-6] However, differentiating oligodendroglial tumors from astrocytomas pathologically may not always be straightforward and it is often subject to interobserver variability, even among experienced neuropathologists.[7] Furthermore, gliomas including oligodendroglial tumors are genetically heterogeneous across patients making them behave and respond to treatment differently, even though they resemble one another histologically.[7] Therefore, identification of genetic markers to confirm pathological diagnosis and to enrich for patients who are likely to respond to therapy has remained a priority in translational neuro-oncology research.[8]

Genetic alterations in oligodendroglial tumors

According to the 2007 World Health Organization (WHO), oligodendroglial tumors can be subdivided into two grades: low-grade (WHO grade 2), which increased cellularity was observed; and anaplastic (WHO grade 3), which increased mitotic activity and nuclear atypia was demonstrated under microscopy.[3] Although classification of glial tumors by the WHO criteria has served neuro-oncology community for several decades, it has been increasingly recognized that histological classification may not be sufficient for diagnosis and prognostication. Understanding molecular and genetic alterations in gliomas has led to not only define the diseases and predict survival outcomes more precisely but also rationally develop the new treatment strategies more effectively.[8] An estimate of 50%-90% of oligodendrogliomas displayed co-deletion (or loss of heterozygosity; LOH) of chromosomes 1p and 19q.[9,10] This co-deletion derived from unbalanced translocation of chromosomes 1 and 19 was found in both low-grade and anaplastic

oligodendroglial tumors.[11,12] Recently, mutations of isocitrate dehydrogenase (IDH) genes have been shown to be the earliest genetic event in a majority of low-grade and anaplastic gliomas.[13,14] IDH, an enzyme critical in tumor cell metabolism, represents the most robust prognostic factor for survival in WHO grade 2-4 gliomas. In addition, there are several studies demonstrating the significant correlation between 1p/19q co-deletion and IDH-1 mutation with virtually all 1p/19q co-deleted oligodendroglial tumors display mutation of either *IDH1* or *IDH2*.[14,15]

Clinical significance of genetic markers in oligodendroglial tumors

As 50%-90% of oligodendrogliomas exhibit co-deletion of 1p/19q, this genetic marker can be used to assist pathological diagnosis in glial tumors with ambiguous histologic phenotypes. Oligodendrogliomas are chemo- and radio-sensitive tumors. Approximately two-thirds of patients with recurrent oligodendrogliomas respond to chemotherapies either PCV (procarbazine, CCNU and vincristine) or temozolomide. Recently, two large randomized phase III trials by the European Organization for Research and Treatment of Cancer (EORTC) and the Radiation Therapy Oncology Group (RTOG) evaluating the role of adjunctive PCV regimen in addition to conventional radiotherapy in patients with anaplastic oligodendroglial tumors demonstrated delayed tumor progression and significant prolongation of overall survival in PCV groups who had 1p/19q co-deletion.[16,17] At present, adjuvant PCV with radiotherapy has become a new standard-of-care treatment for anaplastic oligodendroglioma with co-deletion of 1p and 19q. As radiotherapy can be associated with long-term neurotoxicity, upfront chemotherapy (i.e. temozolomide) treatment in patients with 1p/19q co-deleted progressive tumors following surgical resection may present an alternative option to delay radiotherapy.[18-20] A large intergroup randomized phase III (CODEL) study is ongoing to evaluate the efficacy of radiotherapy plus PCV, radiotherapy plus temozolomide or upfront temozolomide alone.

At the time of project initiation, the analysis of 1p/19q co-deletion was not widely available in Thailand. Therefore, we proposed to use a fluorescent in situ hybridization (FISH) technology to identify the co-deletion in archival oligodendroglial tumor samples at Siriraj Hospital, Mahidol University. This study would elucidate the estimated frequency of deletions of 1p and/or 19q in oligodendroglial tumors and would determine if these genetic markers can predict survival and therapeutic response in our Thai patient populations as our treatment regimens were different

from those of Western countries. These genetic markers may serve as a powerful tool in aiding pathological diagnosis, survival prognostication and treatment decision for patients with oligodendroglial tumors. This is particularly important in Thailand and resource-poor countries, where standard anti-glioma chemotherapy, temozolomide (Temodal®, MSD, New York, USA) is very costly. Judicious selection of patients to receive chemotherapy based on their tumor genetic profiles predicting favorable response may be more rational and cost-effective than universal chemotherapy administration in unselected patient populations. Furthermore, development of this genetic analysis will lay a strong foundation for future research in both clinical and translational aspects of neuro-oncology in Thailand.

Hypoxia in Oligodendroglial Tumors

Hypoxia (low tissue oxygen tension) is a biological feature often found in necrotic area of gliomas.[21] Approximately 16% of anaplastic oligodendroglial tumors demonstrated necrosis within tumors.[22] Hypoxia in tumor microenvironment is important in promoting cell proliferation, resistance to anti-neoplastic agents, angiogenic drive, and invasion/metastasis.[21] Hypoxia is increased with progression of oligodendroglial tumors.[23] Carbonic anhydrase-9 (CA-9) is a hypoxia-inducible transmembrane enzyme that has recently been shown to be an independent prognostic factor for patients with oligodendroglial tumors.[23,24] Furthermore, high tumor hypoxia scores as determined by high expressions of more than one of the following markers: CA-9, hypoxia-inducible factor (HIF)-1α, and vascular endothelial growth factor (VEGF), was associated with poor treatment outcome in oligodendroglial tumors with 1p deletion.[24] Recently, hypoxia-inducible factor (HIF)-2α, a hypoxia-inducible transcription factor, has been shown to be complementary to CA9 as hypoxia determinants to predict survival outcome after treatment with bevacizumab (a neutralizing VEGF antibody) and irinotecan in malignant astrocytoma patients.[25] In addition to regulating angiogenesis and other malignant phenotypes of cancer, HIF-2α is also important in stem cell program by activating of several transcription factors including Oct-4, c-myc and notch.[26] HIF-2α serves as a marker and a critical target in glioma cancer stem cells, which have recently been demonstrated to mediate angiogenesis[27] (a new blood vessel formation from pre-existing vasculature) and therapeutic resistance.[28-30] As HIF-2α expression has not been studied in oligodendroglial tumors, we proposed to study expression of HIF-2α and co-expression

of HIF-2α/CA-9 for the first time in oligodendroglial tumors. HIF-2α may provide additional prognostic value and may serve as a novel therapeutic target in oligodendroglial tumors.

Objectives

1. To determine the estimated frequency of deletions of 1p and/or 19q and IDH1 mutation in oligodendroglial tumors from Thai patients.
2. To determine whether the deletions of 1p and/or 19q and IDH1 mutation are associated with survival advantage and treatment response in Thai patients with oligodendroglial tumors.
3. To determine whether tumor hypoxic profile (expression of CA-9 and HIF-2α)
    3.1. Predicts survival and treatment response in patients with oligodendroglial tumors;
    3.2. Correlates with deletions of 1p and/or 19q in oligodendroglial tumors.
4. To determine whether IDH1 mutation
    6.1 Correlates with deletions of 1p and/or 19q in oligodendroglial tumors;
    6.2 Correlates with hypoxic markers in oligodendroglial tumors.
    6.3 In combination with 1p/19q co-deletion and hypoxic profile can better predict prognosis and treatment response in Thai patients with oligodendroglioma

Material and methods

Fluorescence In Situ Hybridization (FISH)

We identified an approximate of 50 oligodendroglial tumors in paraffin-embedded samples (between 1998 and 2008) from the tissue archive in the Department of Pathology at Siriraj Hospital, Mahidol University. These archival tumors were re-reviewed by two independent pathologists (T.S. and P.C or K.C.) and graded according to the 2007 WHO classification system. A paraffin-embedded tumor block was selected based on content, including the highest grade component and representation of the predominant histologic phenotype of the individual patient and was placed on tissue microarray (TMA). Fluorescence In Situ Hybridization (FISH) technique was exploited to identify deletions of chromosomes 1p and/or 19q as previously described.[11] Briefly, tumor sections were deparaffinized, dehydrated and pretreated with citrate buffer followed by pepsin digestion. Dual-probe hybridization was performed using the Vysis LSI 1p36/LSI 1q25 and LSI 19q13/ LSI 19q13 dual color probe set (Abbott Molecular, USA) according to the

manufacturer's instruction. A microscope with triple-pass filter was used to assess the number of FISH signals for each locus-specific FISH probe. Approximately 100 non-overlapping nuclei were enumerated per hybridization. We used ranges for normal FISH probe copy number as established by Smith *et al*., which were extensively validated by comparing their FISH data with loss of heterozygosity (LOH) and comparative genomic hybridization (CGH) data.[11]

Immunohistochemistry

All formalin-fixed, paraffin-embedded, oligodendroglial tumors on TMA used in the FISH analysis underwent immunohistochemical (IHC) staining for detection of isocitrate dehydrogenase (IDH)-1 R132H mutation with IDH1 R132H antibody (Dianova, Germany), HIF-2α antibody (Novus Biologicals, USA), and CA9 antibody (Novus Biologicals, USA) with a modified diaminobenzidine-streptavidin technique as previously described. Assessment of reactivity was performed as previously described.[21] A dichotomous scoring system (positive, high reactivity *vs* negative, low/absent reactivity) was used. Unequivocal positive staining of IDH1 R132H or HIF-2α was considered positive for each marker. High reactivity of CA-9 was determined by an immunoreactivity score of 9, which was derived from the most intense (3+) staining multiplied by the highest (3+) distribution score of positive cells in tumor sample. Fisher's exact test was performed to assess associations between several genetic and hypoxic markers.

Patients' clinical data

Clinical parameters were retrospectively collected from medical records after an approval by the Siriraj Institutional Review Board (SIRB approval number 410/2552). Telephone interview were performed for patients who have lost to clinical follow-up. Survival analyses were performed with survival defined as the time between tissue acquisition and patient death. Progression-free survival (PFS) was analyzed in a subset of patients who received radiotherapy. PFS was calculated from the time of treatment initiation to the time of clinical an/or radiographic progression. Kaplan-Meier survival curves were plotted to estimate survival distribution and survival rates of patients subsets defined by genetic markers were compared by log-rank tests. Cox proportional-hazards regression was used for multi-variate analysis. Statistical analysis was performed and graphs were generated with a MedCalc program (Ostend, Belgium). $P < .05$ was considered statistically significant.

Results

Patients

Fifty patients with WHO grade II and III oligodendroglial tumors were identified from the archive of Department of Pathology at Siriraj Hospital. However, only 26 tumor samples were adequate in quality and thus processed into TMA for evaluation by FISH and IHC (Table 1). Among 26 patients, 22 (85%) were WHO grade 2 pure oligodendroglioma and 4 (15%) were WHO grade 3 (anaplastic) pure oligodendroglioma. Eleven (42%) patients received radiotherapy alone, whereas 3 patients had chemotherapy (temozolomide) as monotherapy and one received radiation with concurrent and adjuvant temozolomide. At the time of analysis, six patients were dead, whereas eleven patients were still alive and nine patients were loss to follow-up.

Table 1. Patient characteristics

Characteristic	Patients (N=26)
Sex	
Male	10 (38%)
Female	16 (62%)
Age	
Median (range)	35 (20-61)
WHO grade at original diagnosis	
WHO grade 2	22 (85%)
WHO grade 3	4 (15%)
Chemotherapy	4 (15%)
Radiotherapy	12 (46%)

Biomarkers

Representative images of normal and deletion of 1p by FISH are illustrated in Figure 1 and those of IDH1 R132H mutation, CA-9 and HIF-2α are shown in Figure 2. Among 26 patients with oligodendroglioma, the frequencies of deletion of 1p, 19q and 1p/19q were 65%, 85%, and 62%, respectively. Mutation of IDH1 R132H was found in 65% by IHC. High expression of CA-9 was demonstrated in 31% of patients, whereas positive HIF-2α was seen in 77% (Table 2).

Table 2. Expression of biomarkers in 26 patients with oligodendroglioma

Biomarker	Present/Positive	Absent/Negative	Total N
1p deletion	17 (65%)	9 (35%)	26
19q deletion	22 (85%)	4 (15%)	
1p and 19q deletions	16 (62%)	10 (38%)	
IDH1 R132H mutation	17 (65%)	9 (35%)	
CA-9	8 (31%)	18 (69%)	
HIF-2α	20 (77%)	6 (23%)	

Association Between Biomarkers

Fisher's exact test was used to evaluate association between biomarkers (Table 3). Significant association was noted between CA-9 and 1p (p = 0.023) or 1p/19q co-deletion (p = 0.009). Interestingly, high expression of CA-9 identifies 50% of tumors with 1p/19q co-deletion with 100% specificity, 100% positive predictive value and 56% negative predictive value (Table 4).

Table 3. Fisher's exact test (*P* value) for association between biomarkers

Biomarker (N=26)	1p deletion	19q deletion	1p/19q deletion	IDH1 R132H	CA-9	HIF-2α
1p deletion	-	0.1	0	0.42	**0.023**	0.14
19q deletion	0.1	-	**0.014**	0.6	0.28	0.54
1p/19q deletions	0	**0.014**	-	1.0	**0.009**	0.163
IDH1 R132H mutation	0.42	0.6	1.0	-	0.38	1.0
CA-9	**0.023**	0.28	**0.009**	0.38	-	0.13
HIF-2α	0.14	0.54	0.163	1.0	0.13	-

Two-sided $P < 0.05$, significant association

Table 4. Univariate analysis of CA-9 predicting co-deletion of 1p/19q

CA-9	Co-deletion of 1p/19q		Total
	Yes	No	
Positive	8	0	8
Negative	8	10	18
Total	16	10	26

*Two-tailed *P* = .0095 (Fisher's exact test)

Biomarkers and Survival Outcomes

The median survival of patients with co-deletion of 1p and 19q was not reached, whereas the median survival for non-co-deleted patients was 55 months (Hazard Ratio, HR = 9.2; 95% CI 1.7, 49; Figure 3A, P = 0.01, log-rank test). Among 11 evaluable patients who received radiotherapy, 7 had co-deletion of 1p and 19q. Co-deletion of 1p and 19q was associated with better progression-free survival, when compared with non-co-deleted tumors (HR 3.8; 95% CI 0.3 – 48; Figure 3B, P = 0.04, log-rank test). Similarly, IDH1 R132H mutation was associated with a trend towards significance of improve overall survival (Figure 4A, P = 0.09, log-rank test). Six of out eleven patients who underwent radiotherapy displayed IDH1 R132H mutation in their tumors. The median PFS following radiation for IDH1 R132H-mutant tumors was 31 months, whereas that for non-IDH1 R132H-mutant tumors was 17 months (HR 0.21; 95% CI 0.05 – 1.02; Figure 4B, P = 0.03, log-rank test). High expression of CA-9 was associated with longer overall survival (median survival, not reached), when compared with no or low CA-9 expression (median survival 55 months) (Figure 5A, P = 0.039, log-rank test). Among 1p/19q co-deleted tumors, high CA-9 expression is also associated with longer overall survival. HIF-2α expression was not associated survival outcomes (Figure 5B). In addition, PFS following radiation treatment was not influenced by expression of either CA-9 or HIF-2α (data not shown)**.**

Age > 50 is a prognostic factor for overall survival (P = 0.008, log-rank test) and PFS following radiation (age, P = 0.0004, log-rank test), whereas WHO grade serves as a prognostic factor for only overall survival (P 0.008, log-rank test). Cox proportional-hazard regression revealed that only 1p/19q co-deletion remained a significant (P = 0.04) prognostic factor for overall survival when including age or WHO grade.

Discussion

Oligodendrogliomas are more sensitive to chemotherapy and radiation, when compared with other gliomas in adults.[5,6] Approximately two-thirds of patients with recurrent oligodendrogliomas respond to chemotherapies either PCV (procarbazine, CCNU and vincristine) or temozolomide.[6] Durable responses to radiation and chemotherapy have been observed in patients with oligodendroglial tumors harboring the co-deletion.[6,9] Recently, two large randomized phase III trials demonstrated delayed tumor progression and prolonged median survival in anaplastic

oligodendroglioma patients with 1p/19q co-deletion that received PCV regimen.[16,17] Therefore, adjunctive chemotherapy, particularly PCV, has been a new standard-of-care treatment for co-deleted anaplastic oligodendroglial tumors since 2012. At present, co-deletion of 1p/19q is routinely used by oncologists to guide treatment for anaplastic oligodendroglial tumors. Furthermore, as radiotherapy can be associated with long-term neurotoxicity in approximately one-third of long-term survivors, upfront chemotherapy treatment in patients with 1p/19q co-deleted progressive oligodendroglial tumors for both low-grade (WHO grade 2) and high-grade (WHO grade 3) following surgical resection may present an alternative option to prevent premature cognitive deficit from early radiotherapy.[18-20]

To our knowledge, this is the first study to perform analysis of 1p/19q co-deletion in oligodendroglial tumors in Thailand. We exploited FISH technology to identify the co-deletion. The frequency of 1p/19q co-deletion in our oligodendroglial tumors is comparable to those reported in the literature and it is similarly associated with better survival and response to radiation treatment in our Thai patient populations. As the majority of our patients received no chemotherapy, the predictive value of chemotherapy benefit cannot be assessed. IDH1 R132H mutation, the most common form of IDH mutation in gliomas, is found in 65% of our patients and it is associated with improved outcomes in a univariate analysis. Previous studies showed that virtually all 1p/19q co-deleted tumors contained IDH1 or IDH2 mutations. In contrast, our study failed to demonstrate correlation between IDH1 R132H mutation and 1p/19q co-deletion. Explanations for this discrepancy may include small sample size and analysis of only a subtype of IDH1 mutation (R132H) that accounts for approximately 90% of all IDH mutations. Future studies using gene sequencing to detect all IDH1 and IDH2 mutations in our larger cohorts are warranted.

Interestingly, high expression of CA-9 identifies the co-deletion of 1p and 19q with specificity of 100% and sensitivity of 50%. This subset of 1p/19q-co-deleted oligodendroglioma with high CA-9 expression has a better prognosis than co-deleted tumors with no or low CA-9 expression. If independently validated, CA-9 may represent a simple test to screen for co-deletion of 1p/19q, particularly in resource-poor settings. If CA-9 is highly expressed, evaluation by more costly FISH, LOH analysis or CGH to detect the co-deletion may not be required. In contrast, if CA-9 expression is low or negative, testing for co-deletion is still indicated given the relatively low sensitivity of CA-9 to predict the co-deletion. CA-9 may present a new therapeutic target in a subset of 1p/19q-co-deleted oligodendroglioma as it is a transmembrane protein, which can be

targeted by antibody or small molecule inhibitors. Prior studies demonstrated that CA-9 expression was associated with poor survival in oligodendroglial tumors. Järvelä et al. reported that CA-9 is associated with poor prognosis in oligodendroglioma and there was no correlation between CA-9 and co-deletion of 1p/19q.[23] Birner and colleagues demonstrated that high hypoxic score as defined by two out of three positive expression of HIF-1α, CA-9 or vascular endothelial growth factor-mRNA in 1p-deleted oligodendroglioma portended poor outcomes in patients who received adjuvant therapy.[24] Our study showed that high expression of CA-9 is associated with improved overall survival and is not predictive of poor outcome following radiotherapy. The discrepancy may be derived from the difference in interpretation and scoring system for CA-9 expression. High expression of CA-9 in our study is defined by the most intense staining combined with the highest distribution score of positive cells. In addition, most oligodendroglial tumors in our study are low-grade (WHO grade 2) pure oligodendrolgioma and are primary (newly diagnosed) in nature. In contrast, prior studies included a significant number of anaplastic (WHO grade 3), mixed oligoastrocytoma and recurrent tumors, which might confer the differences in biologic characteristics and degree of hypoxia. Small sample size in our study may also have an influence on the findings. Our studies have other limitations including retrospective nature of the study and loss to follow-up of several patients. We advocate prospective and independent studies from others to confirm our findings.

As a consequence of this study, these genetic markers now serve as an important tool in aiding pathological diagnosis, survival prognostication and treatment decision for patients with oligodendroglial tumors at our institution. In addition, this experiment has laid a strong foundation for ongoing research in both clinical and translational aspects of neuro-oncology at Siriraj Hospital as well as other centers in Thailand.

Conclusions: Co-deletion of 1p and 19q and IDH1 R132H mutation are present in 62% and 65% , respectively, in our cohort of Thai patients with oligodendroglioma. Both biomarkers correlate with favorable survival outcomes. High tumor expression of CA-9, a marker for hypoxia and acidosis, identifies a subset of oligodendrogliomas with co-deletion of 1p and 19q and indicates better survival.

Future Directions: Validation of the association between CA-9 and co-deletions of 1p/19q in larger patient populations and in other research groups is required. Upon validation, CA-9 testing may represent a simple screening for co-deletion of 1p/19q and may serve as a new therapeutic

target for a subset of co-deleted oligodendrogliomas. In addition, molecular mechanism underlying the relationship between hypoxia/acidosis and co-deletion of 1p/19q should be explored.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกอ. และ สกว.

1. **Manuscript**

   High expression of carbonic anhydrase-9 identifies a subset of 1p/19q-co-deleted oligodendroglial tumors with favorable prognosis.

   Sith Sathornsumetee, Pornsuk Chuensochol, Komkrit Changkaew, Tumtip Saengruchi

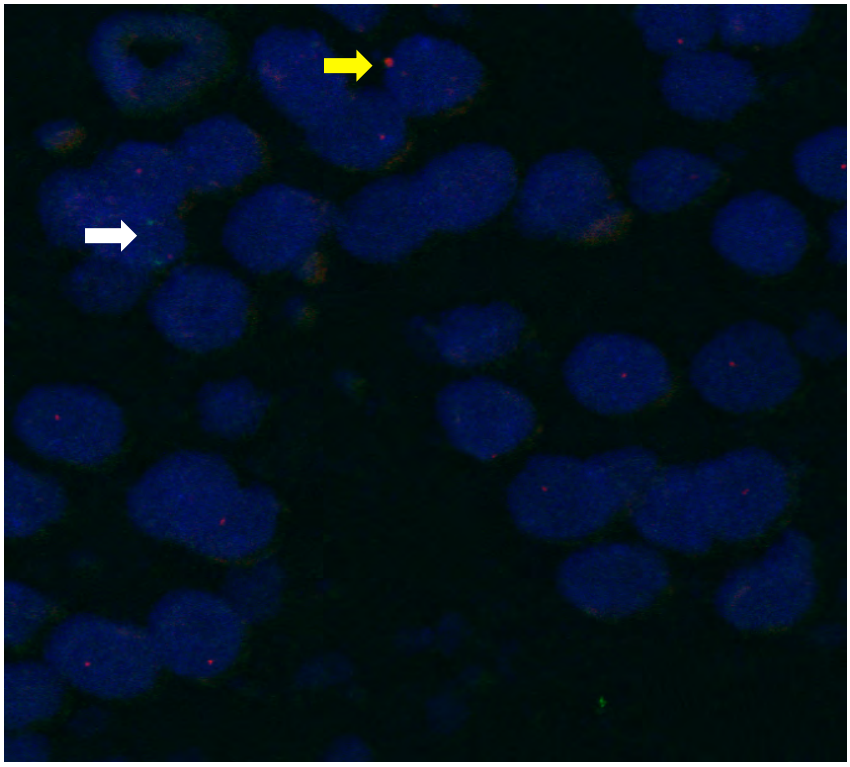
   Manuscript in Preparation

2. การนำผลงานวิจัยไปใช้ประโยชน์

   คณะแพทยศาสตร์ศิริราชพยาบาลได้จัดการตรวจเพื่อหาความผิดปกติทางพันธุกรรม deletion ของโครโมโซม 1p และ 19q ในเนื้องอกชนิด oligodendroglioma เพื่อช่วยวินิจฉัยทางพยาธิวิทยา ช่วยบอกการพยากรณ์โรคและช่วยตัดสินใจในการรักษาผู้ป่วยด้วยยาเคมีบำบัด ซึ่งจะเป็นประโยชน์สำหรับผู้ป่วยโรคนี้เป็นอย่างยิ่ง ผลการทดลองที่พบว่ามีความสัมพันธ์ระหว่าง CA-9 และ co-deletion ของ 1p/19q นั้นเป็นสิ่งใหม่และอาจนำไปสู่ความเข้าใจทางด้านพยาธิกำเนิดและพยาธิสรีรวิทยาของโรคนี้ได้มากขึ้น ซึ่งอาจนำไปสู่การค้นพบการรักษาใหม่ๆ สำหรับเนื้องอกชนิดนี้ในอนาคต นอกจากนี้การตรวจย้อมเพื่อหา hypoxic marker เช่น CA-9 และ HIF-2 alpha เป็นการเสริมสร้างทักษะของผู้วิจัยและบุคลากรของคณะแพทยศาสตร์ศิริราชพยาบาล เพื่อให้มีความชำนาญด้านการตรวจและการแปลผลลักษณะ hypoxia ของเนื้องอกสมองต่อไปในอนาคต

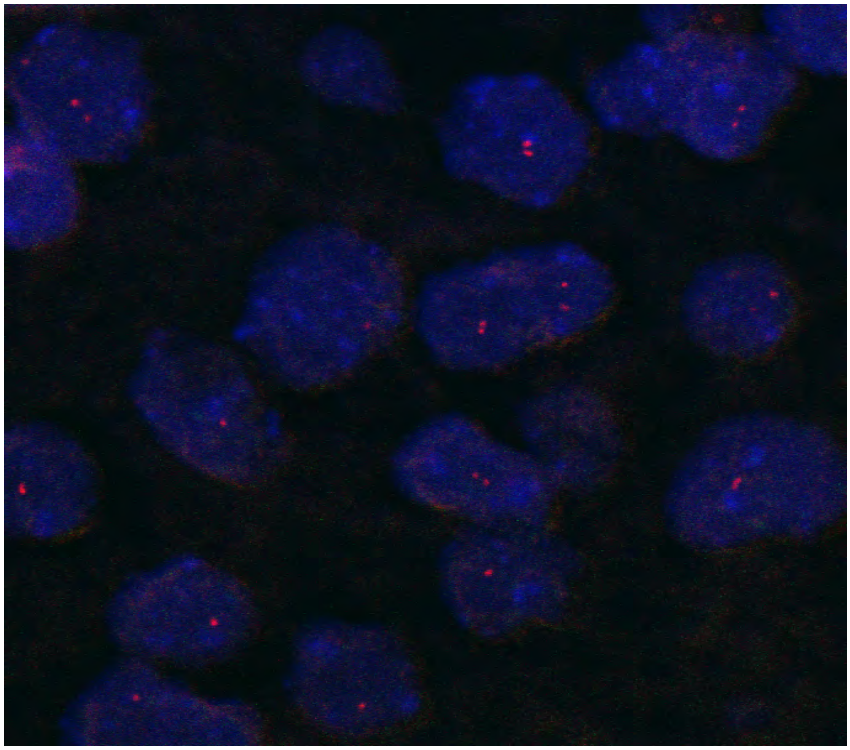
Figure 1.

A



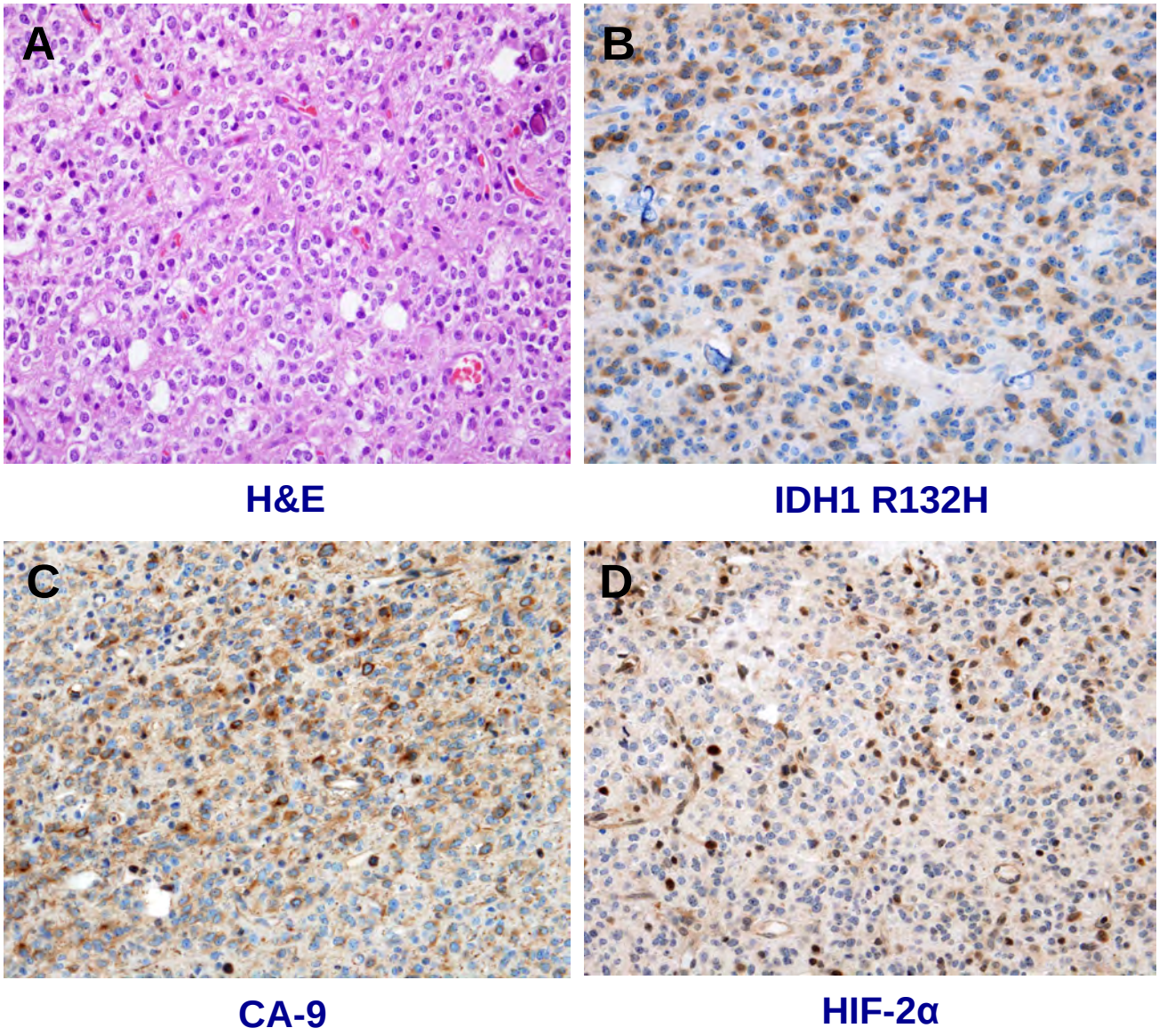
A. Oligodendroglioma with 1p deletion detected by FISH. Confocal microscopy demonstrates that most cells contain one red dot (1p36 probe; yellow arrow) and two green dots (1q25 probe; white arrow)

B



B. Oligodendroglioma with intact 1p detected by FISH. Confocal microscopy demonstrates that most cells contain two red dots (1p36 probe).

Figure 2.



Representative immunohistochemical detection of biomarkers. A. H&E. B. IDH1 R132H mutation staining in tumor cell cytoplasm. C. High (positive) CA-9 expression in tumor cell membrane and cytoplasm. D. Positive HIF-2α expression in tumor cell nuclei and cytoplasm.

Figure 3.

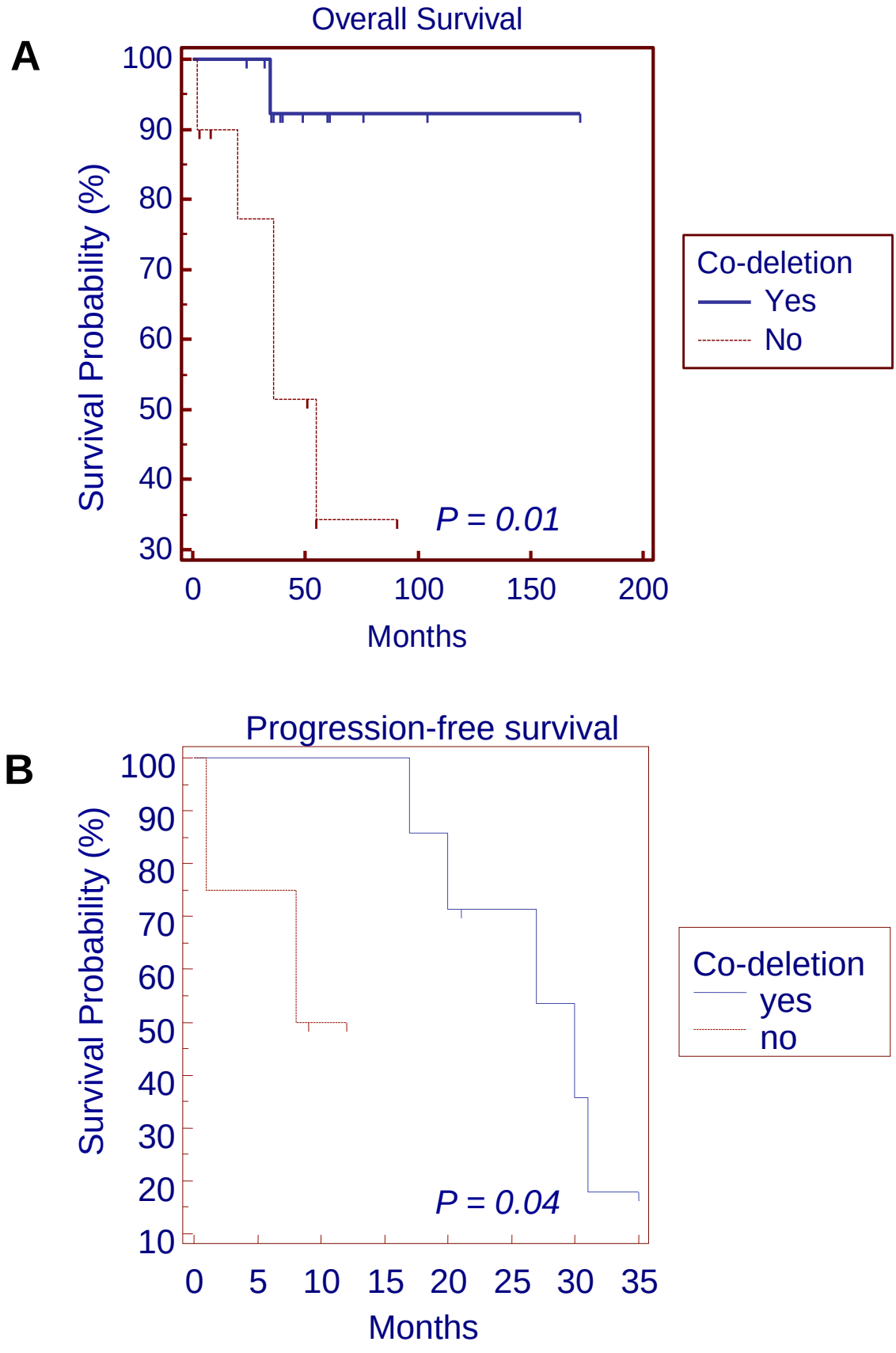
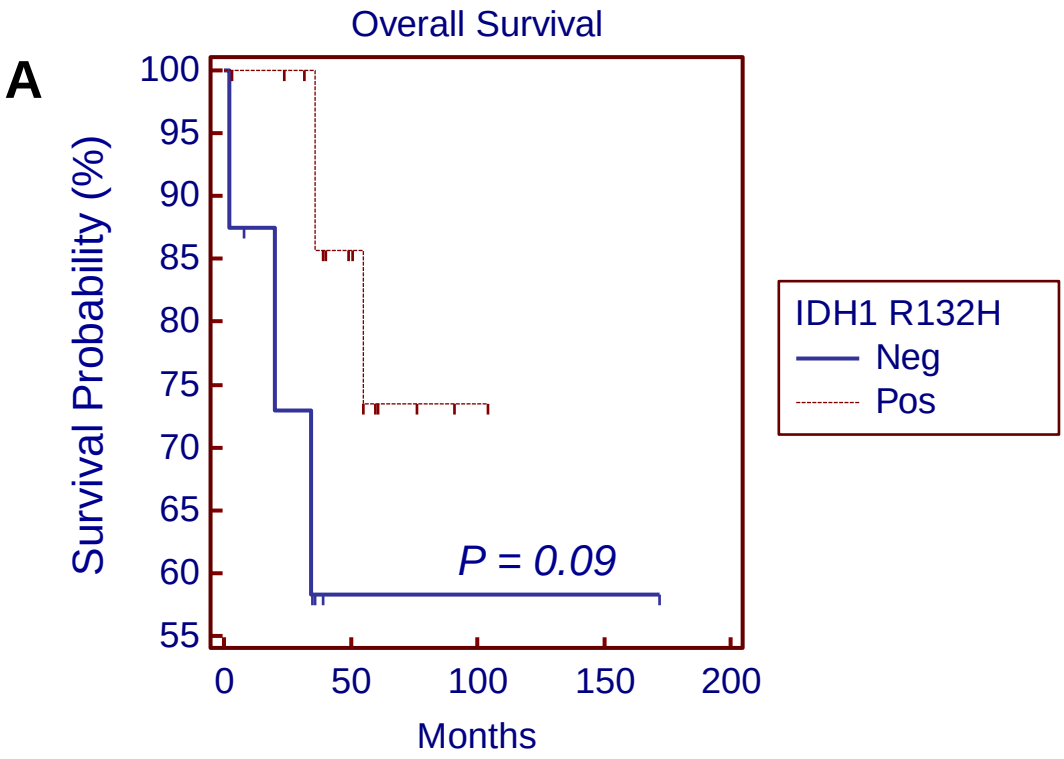

A
Overall Survival
Survival Probability (%)
100
90
80
70
60
50
40
30
0
50
100
150
200
Months
P = 0.01
Co-deletion
Yes
No
B
Progression-free survival
Survival Probability (%)
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
Months
P = 0.04
Co-deletion
yes
no

Figure 4.


A
Overall Survival
Survival Probability (%)
100
95
90
85
80
75
70
65
60
55
0
50
100
150
200
Months
P = 0.09
IDH1 R132H
Neg
Pos

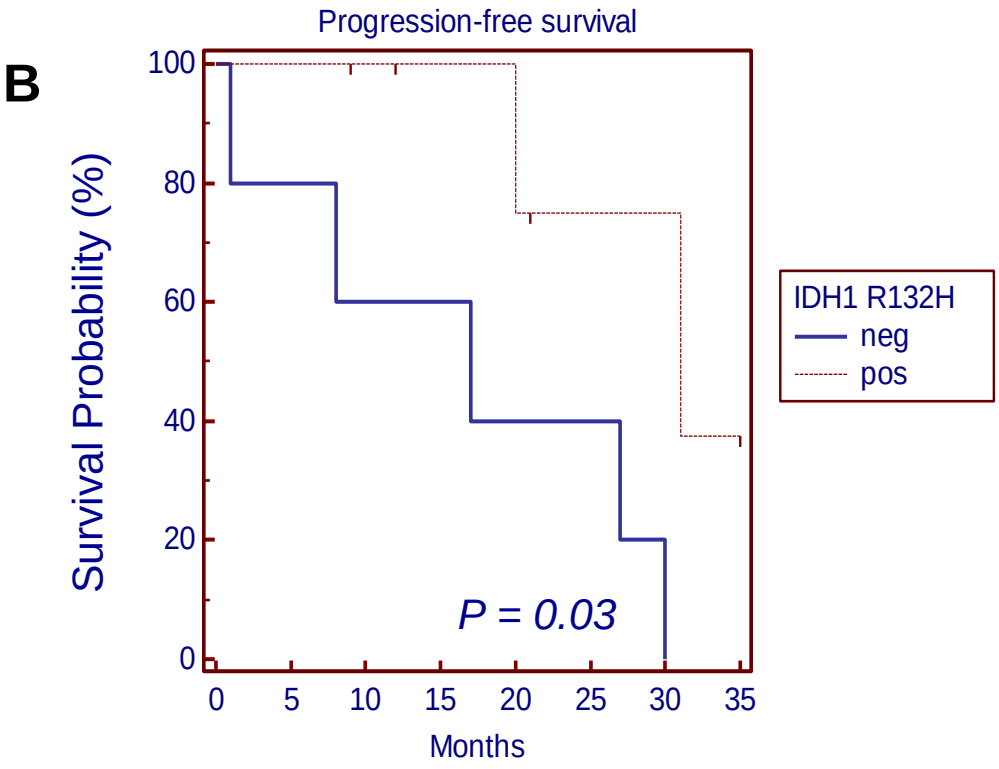
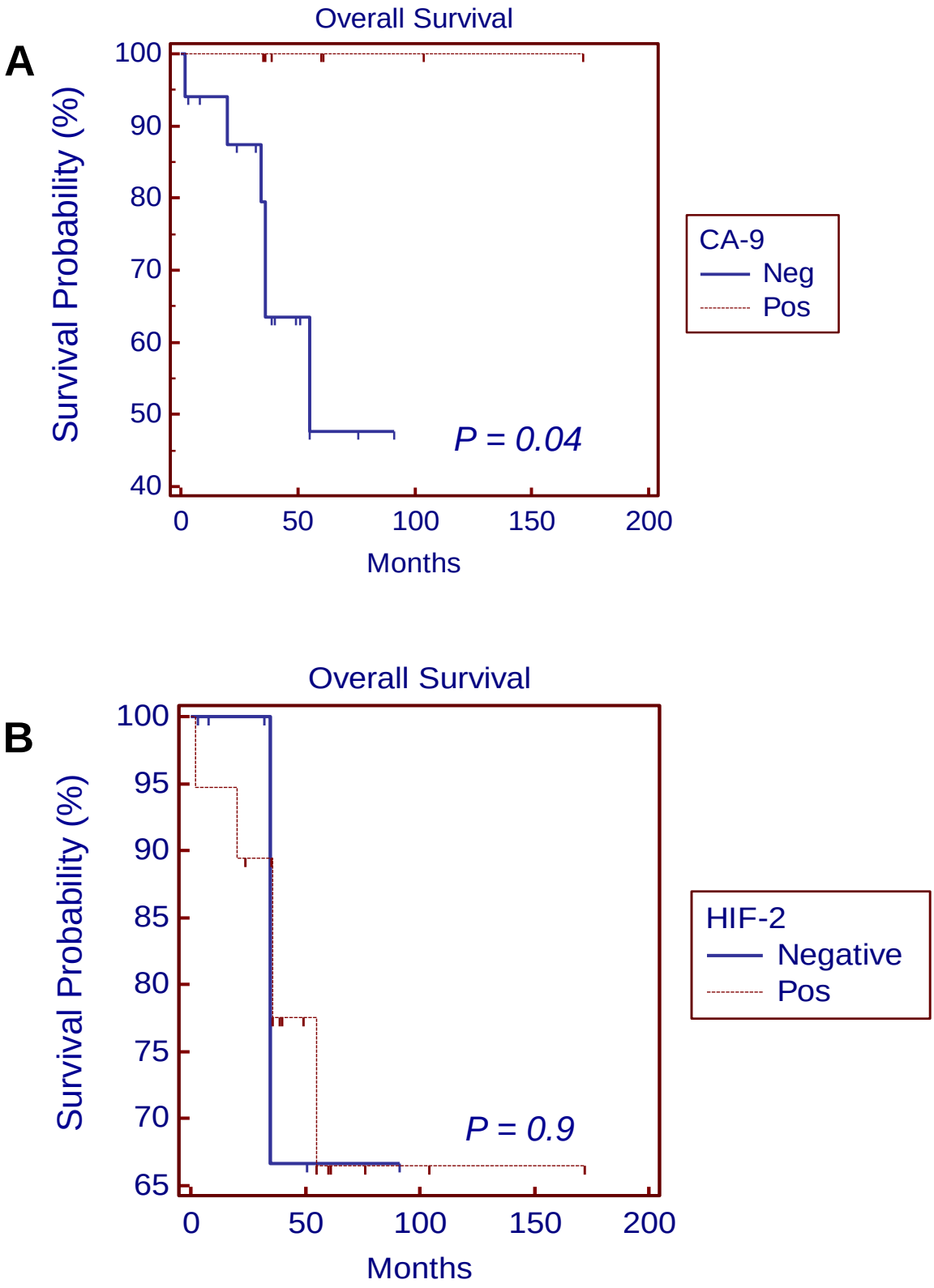

B
Progression-free survival
Survival Probability (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
Months
P = 0.03
IDH1 R132H
neg
pos

Figure 5.


A
Overall Survival
Survival Probability (%)
100
90
80
70
60
50
40
0
50
100
150
200
Months
P = 0.04
CA-9
Neg
Pos
B
Overall Survival
Survival Probability (%)
100
95
90
85
80
75
70
65
0
50
100
150
200
Months
P = 0.9
HIF-2
Negative
Pos