Array-based comparative genomic hybridization identifies CDK4 and FOXM1 alterations as independent predictors of survival in malignant peripheral nerve sheath tumor

Jinsheng Yu¹, Hrishikesh Deshmukh¹, Jacqueline E. Payton¹, Christopher Dunham³, Bernd W. Scheithauer⁴, Tarik Tihan⁵, Richard A. Prayson⁶, Abhijit Guha⁷, Julia A. Bridge⁸, Rosalie E. Ferer⁹, Guy M. Lindberg¹⁰, Rebecca J. Gutmann², Ryan J. Emnett², Lorena Salavaggione¹, David H. Gutmann², Rakesh Nagarajan¹, Mark A. Watson¹, Arie Perry¹ *

Departments of Pathology and Immunology¹ and Neurology², Washington University School of Medicine, Saint Louis, MO, USA; Division of Anatomic Pathology³, Children's and Women's Health Centre of B.C., Vancouver, British Columbia, Canada; Department of Anatomic Pathology⁴, Mayo Clinic, Rochester, MN, USA; Department of Pathology⁵, University of California in San Francisco, San Francisco, CA, USA; Department of Anatomic Pathology⁶, Cleveland Clinic Foundation, Cleveland, OH, USA; Division of Neurosurgery⁷, University Health Network, Toronto, Ontario, Canada; Department of Orthopaedic Surgery⁸, University of Nebraska Medical Center, Omaha, NE, USA; Department of Clinical Neuroscience⁹, King's College London, London, UK; Pathologist¹⁰, Caris Diagnostics, Dallas, TX, USA.

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Corresponding author:

Arie Perry, MD

Department of Pathology and Immunology

Washington University School of Medicine

660 South Euclid Avenue, Campus Box 8118

St. Louis, MO 63110, USA

Email: aperry@wustl.edu

Phone: 314-518-2861

Fax: 314-362-7765

* Current address:

Department of Pathology, Division of Neuropathology

University of California, San Francisco (UCSF)

505 Parnassus Avenue, #M551, Box# 0102

San Francisco, CA 94143

Ph# 415-476-5236 or 415-476-4961

Fax# 415-476-7963

E-mail: Arie.Perry@ucsf.edu
STATEMENT OF TRANSLATIONAL RELEVANCE

Malignant peripheral nerve sheath tumor (MPNST) is an aggressive malignancy with considerable biological variability, a variable and often lethal clinical course, and limited response to current therapeutic approaches. A major impediment to more effective disease management has been a lack of available prognostic or predictive biomarkers. Through initial identification of several survival-associated copy-number alterations, followed by confirmatory assays and a comprehensive multivariate analysis, our present study demonstrates that CDK4 gain/amplification and increased FOXM1 protein expression are significant independent predictors for poor survival in MPNST patients. If confirmed in additional prospective studies, these biomarkers may provide clinically useful information for managing patients with this aggressive malignancy. Interestingly, a previous report has demonstrated that the multi-kinase inhibitor sorafenib can inhibit MPNST growth, suggesting the potential of CDK4 as a therapeutic target and also as a potential predictive biomarker of response to sorafenib therapy.
Abstract

**Purpose:** Malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive sarcomas with variable patient survival and few known prognostically relevant genomic biomarkers. To identify survival-associated genomic biomarkers, we performed high resolution array-based comparative genomic hybridization (aCGH) on a large set of MPNSTs.

**Experimental Design:** Candidate gene alterations identified by aCGH in 38 MPNSTs were validated at the DNA, RNA, and protein levels on these same tumors and an independent set of 87 MPNST specimens.

**Results:** aCGH revealed highly complex copy-number alterations, including both previously reported and completely novel loci. Four regions of copy-number gain were associated with poor patient survival. Candidate genes in these regions include SOX5 (12p12.1), NOL1 and MLF2 (12p13.31), FOXM1 and FKBP1 (12p13.33), and CDK4 and TSPAN31 (12q14.1). Alterations of these candidate genes and several others of interest (ERBB2, MYC and TP53) were confirmed by at least one complementary methodology, including DNA and mRNA quantitative PCR, mRNA expression profiling, and tissue microarray-based fluorescence in situ hybridization and immunohistochemistry. Multivariate analysis showed that CDK4 gain/amplification and increased FOXM1 protein expression were the most significant independent predictors for poor survival in MPNST patients (p<0.05).

**Conclusions:** Our study provides new and independently confirmed candidate genes that could serve as genomic biomarkers for overall survival in MPNST patients.
INTRODUCTION

Malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive and frequently lethal Schwann cell-derived neoplasms associated with poor survival (1-5). Roughly half of MPNSTs develop sporadically and the other half arise in the setting of neurofibromatosis type 1 (NF1). The five year survival rate for NF1-associated MPNST patients is reportedly half of that for sporadic cases (21% vs. 42%) (5). However, other studies suggest that a diagnosis of NF1 does not affect survival (6). Clinicopathologic factors, such as tumor grade and anatomic site reportedly influence MPNST patient outcome, although the clinical course remains highly variable and difficult to predict in individual patients.

Traditional cytogenetic studies have reported that MPNSTs have remarkably complex karyotypes with a wide spectrum of chromosomal alterations including translocations, duplications, and numerical gains and losses, making it difficult to discern which of these alterations are biologically relevant (7-9). Unfortunately, no single alteration has consistently proven to be diagnostically or prognostically useful across multiple studies. For example, alterations on chromosome 17 (where the NF1 gene is located) are expected, given that MPNSTs are highly over-represented among NF1 patients. However, NF1 losses are not always encountered (8-10), suggesting that other tumorigenic pathways also exist. A major limitation of traditional cytogenetic studies, however, has been their limited resolution of ~10 Mb. This has complicated the discovery of specific, recurring genetic alterations involving small regions.

High-resolution genome-wide DNA microarray analyses have identified many novel tumor-specific microdeletions and amplifications in leukemia, lung, and ovarian
cancers (11-13). To date, a similar approach applied to MPNSTs has been limited to few studies with either less comprehensive or lower resolution array platforms (~1 Mb) (14-16). In the present study, we have analyzed a larger cohort of MPNSTs utilizing both Affymetrix and Nimblegen array-based comparative genome hybridization platforms (0.25-2.5 kb of resolution with whole genome coverage) in order to identify recurrent DNA copy-number alterations (CNAs) that are associated with reduced patient survival, independent of traditional clinical and pathological parameters in patients with this extremely heterogeneous tumor. The results from this study provide a short list of candidate genes with CNAs which may prove clinically useful as prognostic biomarkers for patients with MPNST.

MATERIALS AND METHODS

Patients and specimens

A cohort of 38 patients with histologically-confirmed MPNST was included in this study for microarray analysis. Among the 38 patients, 23 (60%) individuals carried a previous diagnosis of Neurofibromatosis Type I (NF1) and 15 (40%) were sporadic cases. At the time of last follow-up, 14 (37%) cases had documented metastases while 16 (42%) had no evidence of metastatic disease. Recurrence data was unavailable for 8 of the cases. The majority (33/38) of cases subjected to genomic analysis were primary tumors, although 5 cases of recurrent tumors were also included. A full accounting of clinical and pathological data is provided in Supplemental Table T1. DNAs and RNAs were extracted from frozen tumors, using commercial reagents (QIAamp DNA Mini Kit, Qiagen; Trizol reagent, Invitrogen) and following manufacturers' standard protocols.
These same specimens were previously utilized for gene expression profiling (17). For biomarker validation studies, another independent set of 121 formalin-fixed paraffin embedded MPNST tumors represented in a tissue microarray (TMA) was utilized; 87 of these patients had clinical annotation and follow up data (18). Of these 87 annotated cases 25 (29%) were NF1-associated and 62 (71%) were sporadic MPNSTs. A total of 68 primary, 12 locally recurrent, and 7 metastatic tumors were represented. The majority of the tumors (78/87) were high grade and 9/87 were classified as low grade. Supplemental Table T2 provides additional data regarding the validation set. All tissue specimens and clinical data from these patients were collected and utilized in accordance with IRB-approved protocols.

**Array-based comparative genome hybridization (aCGH)**

The Affymetrix Genome-wide 500K SNP Mapping array set was employed to detect genomic alterations in the set of 38 MPNSTs. For aCGH analysis, relative copy-number values of the MPNSTs were generated using a reference set of 37 non-malignant DNA samples derived from 22 males and 15 females. To identify meaningful CNAs, two DNA copy-number analysis tools, Partek Genomics Suite (PARTEK, www.partek.com) and Copy-Number Analyzer for GeneChip (CNAG, www.genome.umin.jp) (19) were applied to the aCGH data. CNAs identified with each of the two tools were compared to identify recurrent regions with minimal overlap. Physical positions of CNAs were annotated according to human genome build 35 (www.ncbi.nlm.nih.gov). All CNAs were filtered against the human normal copy-number variation (CNV) database (http://projects.tcag.ca/variation) prior to further analysis. CNAs identified on the 500K SNP platform were also confirmed in a subset of 8 patients where patient-matched
tumor and germline DNAs were available, using a dual-color high-density NimbleGen 135K aCGH platform (see Supplemental Text for details).

**Quantitative real-time PCR (qPCR)**

SYBR Green chemistry was utilized in qPCR assays; qPCR primer sequences are listed in Supplemental Table T3. The qPCR was performed in 10-μl reactions containing 10 ng of cDNA or genomic DNA and gene-specific primers each at 200 nM of final concentration, as previously described (20), on the ABI 7900HT system with the following program: 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 sec, 56 °C for 30 sec, and 72 °C for 30 sec. All sets of primers yielded a single peak in dissociation curve analysis and had an efficiency of greater than 0.9.

**Immunohistochemistry (IHC)**

Fresh tissue microarray (TMA) sections were obtained from the Washington University/Siteman Cancer Center Tissue Procurement Core Facility and IHC was performed using the streptavidin-biotin-peroxidase complex technique as described previously (21). The primary antibodies included FOXM1 (Santa Cruz, Cat. #: sc-502; 1:200 dilution), MYC (Zymed, Cat. #: 9E10; 1:200 dilution), SOX5 (Abcam, Cat. #: ab26041; 1:500 dilution), and p53 (Biogenex, Cat. #: AM240-5M; 1:200 dilution). Evaluation of each core on the TMA was made independently by two pathologists (L.S., A.P.), and the final score for each tumor was derived from the average score of replicate cores on the arrays (see Supplemental Text). CDK4 TMA immunohistochemistry was also performed, but was not interpretable due to poor staining quality of this antibody reagent on the TMA spots.
**Fluorescence in situ hybridization (FISH)**

FISH was performed on TMA slides as previously described (22, 23). Freshly cut slides were subjected to dual-color hybridization with the following probe pairings: centromere enumerating probe 12 (CEP12)/CDK4 (12q14.1), CEP17/HER2 (ERBB2; 17q21.1), and CEP8/MYC (8q24.21), respectively. Five of the probes were obtained commercially and were labeled with SpectrumGreen (CEP8, CEP12, and CEP17) or SpectrumOrange (MYC and ERBB2) fluorochromes (Vysis, Downers Grove, IL). The CDK4 locus was targeted with a locally developed probe, utilizing the RP11-571M6 human BAC clone as previously published (24) (see Supplemental Text).

**Statistical analysis**

Each of the minimally overlapping genomic regions was tested for association with clinical variables, for which the Kaplan-Meier model was used for survival analysis. Differential expression of candidate genes was detected between patients with and without CNAs where positive correlations with survival were seen using the Mann-Whitney U test. Survival probability was first estimated using the Breslow test (univariate Cox regression) for each of the genomic and relevant clinical variables, and then the significant predictors in univariate models were entered into multivariate models. The stepwise selection method was utilized to build the most significant models. The cutoff $P$ value was set as 0.05. All computations were performed in SAS (v9.1.3, Cary, NC).
RESULTS

Characterization of MPNST genomic alterations

As shown by high resolution aCGH (Fig. 1) and further quantified by calculation of the "breakpoint index" (a surrogate measure of genomic instability calculated based upon the total number of independently amplified and deleted genomic segments identified in a tumor based on aCGH analysis, see ref. 13), MPNSTs demonstrate a highly complex and disrupted genome. MPNST genomes had higher overall complexity (mean "breakpoint index"=346) compared to similarly characterized genomes of other tumor types: leukemia (mean=88) (25), ovarian cancer (median=95) (13), and pilocytic astrocytoma (mean=111) (20). The indices were, however, similar to lymphoma (mean=374) (26). The percentage of the total genome affected by CNAs was also calculated to assess genome complexity of individual tumors. This ranged widely from 0.2% to 97% (median 34%) among the 38 MPNSTs (Fig. 1C). This observation no doubt reflects the pathological diversity of tumors characterized in this cohort, although a direct association between reduced survival time and overall high genome complexity (defined here as ≥ 34% of the genome affected by CNA) did not quite reach statistical significance (Fig. 2A, p=0.071).

Many of the genomic loci associated with recurrent copy number alterations harbor genes previously implicated in MPNST biology and were observed with frequencies comparable to those reported in previous studies (14) (Supplemental Table T4). For example, 19 of 38 (50%) cases had deletion at the NF1 locus. Interestingly, however, this event was independent of clinical diagnosis as 13 of 23 NF1 patients (56%) as well as 6 of 15 sporadic cases (40%) demonstrated deletions. Several other
recurrent copy number alterations of genes not previously associated with MPNSTs were also identified. These included gains of \textit{PDCD1} at 2p25.3 (47\% of cases), \textit{SOX5} at 12p12.1 (34\% of cases), \textit{FOXM1} at 12p13.33 (29\% of cases) and \textit{NOL1} at 12p13.31 (29\% of cases) as well as losses of \textit{PSIP1/p75} at 9p22.3 (50\% of cases) (27), \textit{PTPRD} at 9p23-24.1 (47\% of cases) (28) and \textit{PTEN} at 10q23.31 (34\% cases). Although it is likely that NF1-associated and sporadic MPNSTs arise in the context of different genetic backgrounds, we found that none of these nor any other loci identified in this analysis was present with statistical significance between NF1 associated and sporadic MPNSTs.

**Identification of survival-associated genomic alterations**

Minimally overlapping regions of genome alteration (1564 copy-number gains and 252 copy-number losses) identified using two alternate segment calling algorithms were correlated with clinical variables. Each genome region evaluated contained at least one annotated gene and recurred in greater than 10\% of 38 tumors. A detection frequency of 10\% (4 tumors) was chosen as a minimum cut-off to provide sufficient statistical power given the sample size and recognizing the already extreme diversity of this tumor type. Using either Mann-Whitney U test or Chi-square test, no significant association was observed between these regions and clinical variables such as age, gender, tumor status (i.e., primary or recurrent), anatomic location and grade, metastasis, recurrence, or NF1 association. Using the Kaplan-Meier model however, 36 minimally overlapping regions with copy-number gains demonstrated significant associations with decreased survival times (Supplemental Table T5). No regions with copy-number losses were statistically associated with patient survival.
When ranked by both frequency and p-value, the most significant region of copy number gain was a 173kb minimally overlapping region at 12p12.1 that includes only a portion of the SOX5 gene (Fig. 2B). This region demonstrated copy-number gains in 13 tumors, but with variability in the size of the locus affected (Fig. 3A). Three other genomic regions associated with survival were also identified (Fig. 2C-2E), where differential mRNA expression was seen in at least one overlapping gene for each of the regions, based on previously published expression profiling data of the same tumors (Table 1) (17). The sizes of these three regions were 265kb at 12p13.31, 551kb at 12p13.33, and 420kb at 12q14.1, and included the entire coding sequence of NOL1/MLF2 (9 cases; 25%), FOXM1/FKBP4 (11 cases; 31%), and CDK4/TSPAN31 (9 cases; 25%), respectively (Fig. 3B-3D). In comparison, the 8q24.21 locus harboring MYC, a gene frequently amplified in neuroectodermal tumors, was also frequently amplified in 13 (34%) MPNSTs (Fig. 3E) and was associated with a trend toward decreased survival (p>0.05). As described below, this association reached statistical significance on subsequent confirmation experiments that evaluated MYC protein expression (Fig. 2G).

Confirmation of copy-number alterations associated with survival

To confirm results obtained from aCGH data sets, qPCR was performed to quantify DNA copy number for 7 specific candidate genes using the same 38 MPNST DNAs employed for aCGH analysis. DNA qPCR results confirmed associations between decreased survival and DNA copy-number gains for all 7 genes (Table 1). Using the same MPNST RNAs from our previously reported gene expression microarray study (17), qRT-PCR analysis confirmed differential expression for 5 of the 7 genes (NOL1,
MLF2, FOXM1, FKBP4, and CDK4), whereas SOX5 and TSPAN31 showed no differential expression (Table 1). Given that only a portion of the SOX5 gene was involved in the amplified region, we questioned whether this amplification reflected a qualitative rearrangement of the locus, rather than SOX5 amplification. However, sequencing of RNAs from tumors that exhibited SOX5 amplification using both 3' and 5' RACE (rapid amplification of cDNA ends) failed to identify a novel fusion gene transcript in these tumors (data not shown).

We also employed an independent cohort of 121 patients tumors (87 with clinical follow-up) to construct a tissue microarray and evaluate gene amplification and protein expression of several candidate genes (CDK4, FOXM1 and SOX5) that were identified and confirmed by the genomic approaches described above. For comparison, we also examined several other previously reported biomarkers of survival (ERBB2, MYC and TP53) in this same cohort. Using Kaplan-Meier survival analysis, CDK4 amplification in 12 of 79 (15%) cases (scored as both true gene amplification and high-level polysomies/gains by FISH analysis) and increased protein expression of FOXM1 and MYC were statistically associated with significantly shorter overall survival in this cohort (Fig. 2G-2I and Fig. 4). In contrast, MYC and ERBB2 gene amplification as well as TP53 (Fig. 2J) and SOX5 protein immunoreactivity were not associated with overall survival.

Finally, a multivariate analysis was carried out to determine whether any of the genomic biomarkers identified could serve as independent predictors for survival (Table 2). Among all genomic and clinical variables examined, only 4 CNA regions (representatively named as CDK4, FOXM1, NOL1, and SOX5) and anatomic site (Fig.
showed significant associations with survival time by univariate regression analysis in the 38 MPNSTs. In addition, CDK4 gene amplification by FISH analysis, FOXM1 and MYC protein expression, tumor type (i.e., primary, recurrent, or metastatic) (Fig. 2K) and tumor grade (Fig. 2L) also demonstrated statistically significant association with overall survival. In multivariate analysis, however, the most significant survival predictor was CDK4 copy-number gain by aCGH in the 38 patient cohort and corresponding CDK4 gene amplification by FISH in the 87 patient cohort. Tumor anatomic site in the 38 patient cohort, and high FOXM1 protein expression and tumor type in the 87 patient cohort were also independently significant in multivariate analyses.

**DISCUSSION**

MPNST is an aggressive malignancy with considerable biological variability and limited response to current therapeutic approaches. This phenomenon is well demonstrated by the clinical heterogeneity of patient outcome in the current study, with overall survival ranging from less than a month to over 13 years. A major impediment to more effective disease management has been a lack of available prognostic or predictive biomarkers. While other sarcomas exhibit characteristic translocations that lead to signature fusion transcripts (29), a definitive set of clinically relevant genomic alterations has been difficult to identify for MPNST, likely due to the large number of gross structural genomic aberrations identified in individual tumors.

Our current study provides one of the first global, high-resolution genomic views of MPNST with independent validation of several biologically relevant genes. Not surprisingly, the overall level of genomic complexity is higher than previously observed
by conventional cytogenetic analyses. Identifying the presumably small number of biologically relevant genomic alterations among a large number of secondary changes resulting from genomic instability remains a daunting task. For this reason, it was reassuring that analysis of these highly complex data sets nevertheless identified a number of loci already known to be important in MPNST tumorigenesis and malignant progression. These included losses of $NF1$ on 17q11.2 and $CDKN2A$ on 9p21, as well as common gains on 7p, 8q, 12, and 17q. However, a relatively larger sample size, the use of a high-resolution genomic platform, and correlation with clinical follow-up data in this study identified a number of novel genes whose copy number gain could serve as clinically useful prognostic markers. In contrast to single gene, high-level amplification that is seen at $MYC$ and $EGFR$ loci in some tumor types, aCGH analysis typically identifies larger genomic regions exhibiting more subtle copy number increases. This may be attributed to both the limited dynamic range of microarray-based technology as well as biological heterogeneity of the tumors themselves.

Interestingly, some of the genome copy number alterations that we identified by aCGH were discordant when measured at the level of gene transcription and/or protein expression. For example, $SOX5$ copy number gains were associated with diminished survival times, but increased mRNA and protein levels were not. There are a number of explanations for such discrepancies, including the possibility of translocations that result in biologically meaningful alterations unassociated with increased expression. Although sequencing of $SOX5$ transcripts in tumors with amplification at this locus was performed in this study, our inability to identify chimeric transcripts by this targeted sequencing approach does not entirely exclude this possibility. Conversely, while $MYC$ protein and
RNA expression levels correlated with survival, we did not observe a similar relationship at the genome level, as determined by either FISH or aCGH. This suggests that mechanisms of increased expression at the transcriptional level may be more important for this particular gene. These repeated observations underscore the need for a comprehensive evaluation of all candidate biomarkers at the genome, transcriptome, and protein levels in independent sample sets, particularly as they are being considered as prognostic tools.

For example, in the recent literature, p53 expression has been proposed as an important predictor of survival time in MPNST patients (30, 31). Our data, however, did not confirm this finding at the level of protein expression, gene expression, or genome deletion. Although 50% of MPNSTs had deletions at the TP53 locus in our present study, differential mRNA expression between TP53 deleted and non-deleted tumors was not seen (p>0.05) and TP53 deletion itself did not stratify MPNST patients by survival. Furthermore, in the second cohort of 87 MPNSTs, p53 immunoreactivity was not statistically associated with survival time. One reason for this discrepancy between our current study and previous studies could reflect the presence of p53 substitution mutations that would obviously not be detected by aCGH but that could affect protein expression and stability. However, the analytical and interpretive techniques applied to immunohistochemistry in the current study were similar to those of previous reports and as such, this discrepancy is more difficult to explain. In contrast, we did identify recurrent copy number gains involving loci at 12p13.31 and 12p13.33 that were associated with survival on univariate analyses. Although these two regions did not remain statistically significant in our primary cohort of 38 MPNST patients by
multivariate analysis, we were able to subsequently demonstrate concurrent mRNA and protein overexpression of *FOXM1* in this region. In a second larger cohort of 87 MPNSTs, FOXM1 over-expression by IHC was associated with poor survival, a significance which was retained on multivariate analysis. FOXM1 is a transcription factor that is only expressed in proliferating cells and has critical functions in tumor development and progression (32, 33). Expression has been associated with enhanced invasion and metastasis in pancreatic and prostate cancers (32, 34). Increased expression of FOXM1 also correlates with increased tumorigenicity of cultured glioma cells and in human glioblastomas, its expression levels are inversely correlated with patient survival (35). Our current data suggests that increased FOXM1 protein expression is a significant predictor of poor survival in MPNST patients.

Finally, of the numerous candidate loci and genes identified in this study, *CDK4* appears to be the most clinically and biologically significant. This gene was initially identified as a region at 12q14.1 that demonstrated recurrent copy-number gains associated with patient survival. Of the 12 genes within this locus, only *CDK4* demonstrated concordant copy number gains and mRNA overexpression by qRT-PCR. Furthermore, by FISH analysis in an independent patient cohort, we found a significant association between *CDK4* amplification (15% of tumors) and decreased survival in a multivariate analysis. Studies have demonstrated amplification of the *CDK4* region in many human cancers (36-38), a finding variably associated with tumorigenesis and/or survival. This 12q amplicon has long been recognized for its role in sarcoma tumorigenesis. Interestingly, however, in contrast to what has been reported in dedifferentiated liposarcoma (39), MDM2 co-amplification with CDK4 was uncommon in
MPNSTs analyzed in this study. To our knowledge, our study is the first to provide specific evidence for a significant association between \textit{CDK4} amplification and poor patient survival in MPNSTs. Interestingly, a previous report has demonstrated that the multi-kinase inhibitor sorafenib can inhibit MPNST growth, in part through hypophosphorylation of pRB at CDK4-specific sites \cite{40}, suggesting the potential of CDK4 as a therapeutic target and also as a potential predictive biomarker of response to sorafenib therapy.

In conclusion, \textit{CDK4} gain/amplification and increased FOXM1 protein expression were the most significant independent predictors for poor survival in MPNST patients after accounting for well established clinicopathologic prognostic variables in MPNSTs. If confirmed in prospective studies, these prognostic and potentially predictive biomarkers may provide clinically useful information for managing patients with this aggressive malignancy.

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\textbf{REFERENCES}


**Figure Legends:**

**Figure 1.** Demonstration of high complexity genome alterations in 38 MPNSTs, defined by PARTEK Genomic Segmentation algorithm in aCGH analysis. (A) Heat map of SNP-level copy-number data. Copy-number loss is shown in blue and gain in red on each of 22 autosomal chromosomes. A two-color bar at the top of the map indicates the data range in log2 ratio. “Good outcome” is defined as survival > 24 months, "moderate outcome" - survival 13-24 months (or "indetermined" - lost to follow-up while alive, with recorded survival < 24 months), and "poor outcome" - survival <= 12 months. Note that in general, patients with "poor outcome" had more copy-number variations than patients with "good outcome". (B) Frequency of individual copy-number alterations (CNAs), across all autosomal chromosomes in 38 MPNST patients. Blue represents copy-number losses and red denotes copy-number gains. (C) Distribution of percentage of the total genome affected by CNAs among 38 MPNSTs analyzed by aCGH. Calculation of "percentage" is based upon NCBI Build 35, May 2004 Assembly (hg17) for human genome size (2864255932 bp for autosome).

**Figure 2.** Kaplan-Meier survival curves in MPNST patients of two independent cohorts, stratified by specific genome copy number alterations or clinical variables, as indicated in each panel: (A-F) initial set of 38 MPNSTs, (G-L) confirmation set of 87 MPNSTs. Less than the total number of tumors in each cohort was analyzed when individual biomarker or clinical data was not available.

**Figure 3.** Five minimally overlapping regions of copy-number gain identified by cross-comparison of segmentation data using two independent algorithms in copy-number
analysis of a set of 38 MPNSTs. For each of 38 tumors, two segments are shown in adjacent columns: left – segmentation detected by Hidden Markov Model, right – segmentation detected by Genomic Segmentation algorithm. Categories of copy-number alterations are designated by a gray scale from white (neutral), light-gray (deletion), and dark-gray (low copy-number gain) to black (high-level DNA amplification). The dotted lines in each of the 5 regions indicate the minimally overlapping region in cross-comparison. The genomic location is shown at the top and bottom within each region, along with representative genes at specific cytobands. Patients outcome: "good" - survival > 24 months, "moderate" - survival 13-24 months (or "indetermined" - lost to follow-up while alive, with recorded survival < 24 months), and "poor" - survival <= 12 months.

**Figure 4.** (A-D) Representative TMA FISH analysis of *CDK4* copy-number gain (red, CDK4 probe; green, chromosome 12 probe). (A) *CDK4* gene amplification; (B) High-level polysomy 12; (C) polysomy 12; (D) normal copy-numbers of *CDK4* and chromosome 12. For survival analysis, A and B were grouped into a single category of *CDK4* amplification, with C and D classified as *CDK4* non-amplification. (E-H) Representative TMA IHC analysis of FOXM1 (E, F) and MYC (G, H) protein expression. High expression (E, G; scores of +++ and ++) versus low or negative expression (F, H; scores of + and -) for each antibody is shown.
Research

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* Only counts on cases with evaluable tissue cores in 57 patients who had complete survival data available for further analysis.
Table 1. Validation summary on potential survival marker genes.

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### Table 2. Multivariate analysis of both clinical and genomic variables for independent survival predictors in MPNST patients.

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Notes: Numbers in parenthesis are 95% confidence interval; a – Univariate Breslow method; b – Multivariate Cox regression.
Array-based comparative genomic hybridization identifies CDK4 and FOXM1 alterations as independent predictors of survival in malignant peripheral nerve sheath tumor


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