Received: 13 September 2022 | Accepted: 24 October 2022

DOI: 10.1111/bpa.13132

RESEARCH ARTICLE

Brain Pathology

Clinical implementation of integrated molecular-morphologic risk prediction for meningioma

Thomas Hielscher[1] | **Martin Sill**[2] | **Philipp Sievers**[3] | **Damian Stichel**[3] | **Sebastian Brandner**[4,5] | **David T. W. Jones**[2,6] | **Andreas von Deimling**[3] | **Felix Sahm**[3] | **Sybren L. N. Maas**[7,8]

[1]Department of Biostatistics, German Cancer Research Center (DKFZ), Heidelberg, Germany

[2]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany

[3]Department of Neuropathology, University Hospital Heidelberg and CCU Neuropathology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

[4]Division of Neuropathology, National Hospital for Neurology and Neurosurgery, University College London NHS Foundation Trust, London, UK

[5]Department of Neurodegenerative Disease, Queen Square Institute of Neurology, University College London, London, UK

[6]Division of Pediatric Glioma Research, German Cancer Research Center (DKFZ), Heidelberg, Germany

[7]Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands

[8]Department of Pathology, Erasmus Medical Center, Rotterdam, The Netherlands

Correspondence
Felix Sahm, Department of Neuropathology, University Hospital Heidelberg and Clinical Cooperation Unit Neuropathology (B300), German Cancer Research Center (DKFZ), Im Neuenheimer Feld 224, Heidelberg 69120, Germany.
Email: felix.sahm@med.uni-heidelberg.de

Funding information
Deutsche Krebshilfe, Grant/Award Number: 70112956; Gemeinnützige Hertie-Stiftung

Abstract

Risk prediction for meningioma tumors was until recently almost exclusively based on morphological features of the tumor. To improve risk prediction, multiple models have been established that incorporate morphological and molecular features for an integrated risk prediction score. One such model is the integrated molecular-morphologic meningioma integrated score (IntS), which allocates points to the histological grade, epigenetic methylation family and specific copy-number variations. After publication of the IntS, questions arose in the neuropathological community about the practical and clinical implementation of the IntS, specifically regarding the calling of CNVs, the applicability of the newly available version (v12.5) of the brain tumor classifier and the need for incorporation of *TERT*-promoter and *CDKN2A/B* status analysis in the IntS calculation. To investigate and validate these questions additional analyses of the discovery ($n = 514$), retrospective validation ($n = 184$) and prospective validation ($n = 287$) cohorts used for IntS discovery and validation were performed. Our findings suggest that any loss over 5% of the chromosomal arm suffices for the calling of a CNV, that input from the v12.5 classifier is as good or better than the dedicated meningioma classifier (v2.4) and that there is most likely no need for additional testing for *TERT*-promoter mutations and/or homozygous losses of *CDKN2A/B* when defining the IntS for an individual patient. The findings from this study help facilitate the clinical implementation of IntS-based risk prediction for meningioma patients.

KEYWORDS
brain tumors, meningioma, molecular biomarkers, risk prediction, tumor classification

Felix Sahm and Sybren L.N. Maas contributed equally.

1 | INTRODUCTION

Meningiomas are the most common intracranial tumors in adult patients [1]. Historically, the diagnosis and grading of meningiomas relied on histological examination. As for other central nervous system (CNS) tumors, molecular markers have gradually been introduced for the risk prediction of meningiomas [2]. In the most recent 2021 CNS5 classification of CNS tumors, the presence of *TERT*-promoter mutations [3] and/or the homozygous loss of *CDKN2A/B* [4] have been introduced as a criteria for a CNS WHO grade 3 meningioma regardless of the histological appearance [2]. With the introduction of these markers, molecular testing has been added to the meningioma risk prediction toolkit of neuropathologists. However, these markers are only able to identify a small proportion of high-risk meningiomas and do not stratify between low and intermediate-risk tumors. Outside of the current WHO grading system, other molecular methods have been developed to stratify risk for progression over different strata, including risk prediction for low and intermediate-risk meningiomas. The approaches include epigenetic risk assessment based on commercially available whole genome methylation arrays [5] or stratification based on the presence of specific copy-number variations (CNVs) supplemented by the mitotic count [6]. In 2021 we published an integrated molecular-morphologic meningioma classification based on an integrated score (IntS) that is calculated by points awarded to the histological grade, the epigenetic methylation family (MF) and specific CNVs [7]. The exact calculation of the IntS is explained in Table 1. Information to determine the MF is obtained through the molecularneuropathology.org website. This website provides an online tool to classify central nervous system (CNS) tumors by referencing the epigenetic profile to a cohort of more than 2800 CNS tumors [8]. Results from this so called "brain tumor classifier" are now included as diagnostic criteria in the 2021 CNS5 WHO classification [2]. In addition to the brain tumor classifier that includes the full spectrum of CNS tumors, there is also a separate and dedicated classifier (currently at version 2.4) available to subclassify meningioma MFs based on epigenetic features correlating with different risk levels for progression [5]. So far information from the v2.4 meningioma classifier was used to determine the MF for IntS calculation. Since the introduction of the IntS, we have seen researchers and clinicians adopting the IntS method both for scientific purposes and for the determination of risk for progression in individual meningioma patients. However, several practical questions simultaneously arose regarding the scoring of the CNVs, MF and the adoption of the next version of the brain tumor classifier (v12.5) that became publicly available after the IntS publication. Here, we address these questions to facilitate further clinical adoption of molecular-morphologic meningioma risk prediction.

TABLE 1 Integrated risk score (IntS) calculation method

Histological WHO grade points	Points
Grade 1	0
Grade 2	1
Grade 3	2
Methylation family (class) points	
Benign	0
Intermediate	2
Malignant	4
Losses chromosome 1p, 6q, 14q (CNVs) points	
None present	0
1–2 present	2
3 present	3
Integrated risk score (IntS) total: Histology points + Methylation family points + CNVs points = IntS	**Risk for progression**
0–2	Low
3–5	Intermediate
6–9	High

2 | MATERIALS AND METHODS

All clinical data included in this manuscript was previously published [5, 7]. Briefly, with approval according to local regulators supplemented with patient consent, meningioma tissue and patient data were collected from the archives of collaborating centers in Germany, Switzerland, United States, United Kingdom and Austria [7]. Details regarding the establishment and selection of the discovery, retrospective validation and prospective validation cohorts can be found in the original publication on the IntS [7]. The analyses were divided into a retrospective discovery cohort ($n = 514$), a retrospective validation cohort ($n = 189$) and a prospective validation cohort ($n = 287$). Details regarding the establishment and selection of the discovery, retrospective validation and prospective validation cohorts can be found in the original publication on the IntS [7]. All cohorts were obtained and analyzed independently. For all cases DNA methylation data and CNV-data were available and for a subset of cases panel sequencing data was also available.

Details regarding the development and calculation of the IntS can be found in the original publication [7]. Briefly, L1-penalized (LASSO) Cox regression was applied to identify an optimal combination of CNVs for meningioma risk prediction. To determine the IntS, a multivariable Cox regression model including histological WHO grade, MF and the LASSO-derived CNV model was fitted. For practical applicability, no more than four points were allowed for an individual modality and all values in the nomogram were rounded to the nearest integer.

For the current study, Harrell's c-index and integrated Brier score were used to assess discrimination and

prediction performance of risk models and were tested for differences between models [9, 10]. *p*-values below 0.05 were considered significant. All analyses were carried out using the R software with add-on packages pec and compareC.

3 | RESULTS

The IntS is calculated by the addition of points awarded for the histological WHO grade, the epigenetic MF and the presence of losses in the chromosomal arms of 1p, 6q and 14q (Table 1). The losses of 1p, 6q and 14q were identified by a Lasso Cox model as the optimal combination of CNVs whereas the predictive value of the individual losses in these (and other) chromosomal arms have been described before [11–13]. Thus, with the integration of the morphology, epigenetic status and the chromosomal stability, the IntS combines three independent risk stratifications for meningioma and was shown to outperform prediction models based on one modality (i.e., histology, epigenetics or CNVs) in three independent cohorts [7].

3.1 | Any chromosomal loss is included in the IntS calculation

When studying the presence of a loss in a specific chromosomal arm, multiple possible outcomes can be observed. First, no loss or gain can be observed and thus the arm is considered balanced (Figure 1A). Second, the full chromosome or chromosome arm can be lost and thus a complete loss of the arm is observed. Thirdly, only a section of the chromosomal arm is lost and thus a segmental loss is detected. Lastly, both a segmental loss and a segmental gain could be observed. The loss percentage was determined by the fork (expansion of the original code base, https://github.com/dstichel/conumee/) of the conumee R package for enhanced CNV analysis [14]. We initially binned the losses and gains in increments of 5% and therefore a complete loss was determined when >95% of the chromosomal arm were lost, a segmental loss as 5–95% loss, balanced as <5% loss (to avoid overcalling of losses due to technical noise) and a segmental gain and loss when both a gain and loss between 5% and 95% was present (Figure 1A). When plotting the Kaplan–Meier (KM) plots of the different possible outcomes of a loss in chromosomes 1p, 6q and 14q (Figure 1B–D) it is clear that any extent of loss is associated with an increased risk for progression and thus any combination that contains a loss of >5% of 1p, 6q or 14q is included in the calculation of the IntS or any other model based on CNVs. This approach was also followed in the initial comparison of the different prediction models [7]. Since our study was based on data obtained from HumanMethylation450 (450 k) and EPIC (850 k) chips by Illumina that quantify the presence of DNA fragments by optical intensity methods, copy neutral loss of heterozygosity will not be detected as a loss. The impact of this on the accuracy of the IntS remains to be investigated.

3.2 | Moving the IntS beyond the separate meningioma classifier v2.4

The IntS is based on the results of the meningioma classifier version v2.4 that is available on the molecularneuropathology.org website. This is a dedicated classifier solely tasked with meningioma classification and is based on the results presented in the 2017 publication describing the identification of three methylation families (benign, intermediate and malignant) that are associated with low, intermediate or high risk for progression in meningioma [5]. The benign and intermediate families are further divided into three benign (ben-1, ben-2 and ben-3) methylation classes (MC) and the intermediate family is divided into 2 MCs (int-A and int-B) [5]. For each sample studied, a calibrated prediction score is returned by the classifier. This calibrated score for an MF or MC can be below or above a certain threshold that results in either a "no match" or "match" as defined by the classifier. When defining the IntS, attempts were made to optimize the IntS by attributing more or fewer points in cases in which the calibrated score for the MC or MF was below a certain threshold, but none of these refined models resulted in improved prediction accuracy over the three cohorts studied. Therefore, for the IntS the highest scoring MF (i.e., benign, intermediate or malignant), regardless of the total value of the score, is used for the determination of the points awarded.

In the overarching brain tumor classifier, meningioma tumors were so far not sub-specified. In the first publicly available version of the classifier, only the methylation class meningioma, without subclasses, was included [8]. Therefore, in daily practice, application of the IntS required the assessment of two different classifiers. First, the brain tumor classifier (at the time of IntS publication at version v11b4) is run to confirm that the case is a meningioma, and then subsequently the meningioma classifier is applied to identify the benign, intermediate or malignant MF. Currently, a new version of the classifier (listed on the website as "v12.5" but will be released as "v12") is publicly available that will eventually replace the current v11b4 version. The v12.5 brain tumor classifier includes the meningioma methylation families and meningioma classes as identified in the separate meningioma classifier. Since the v12.5 classifier includes a wide spectrum of CNS tumors with many different types and subtypes of CNS tumors, the nomenclature has changed to accommodate all identified CNS tumor (sub)entities. Meningioma tumors form the meningioma MF and are further subdivided into methylation classes. These classes

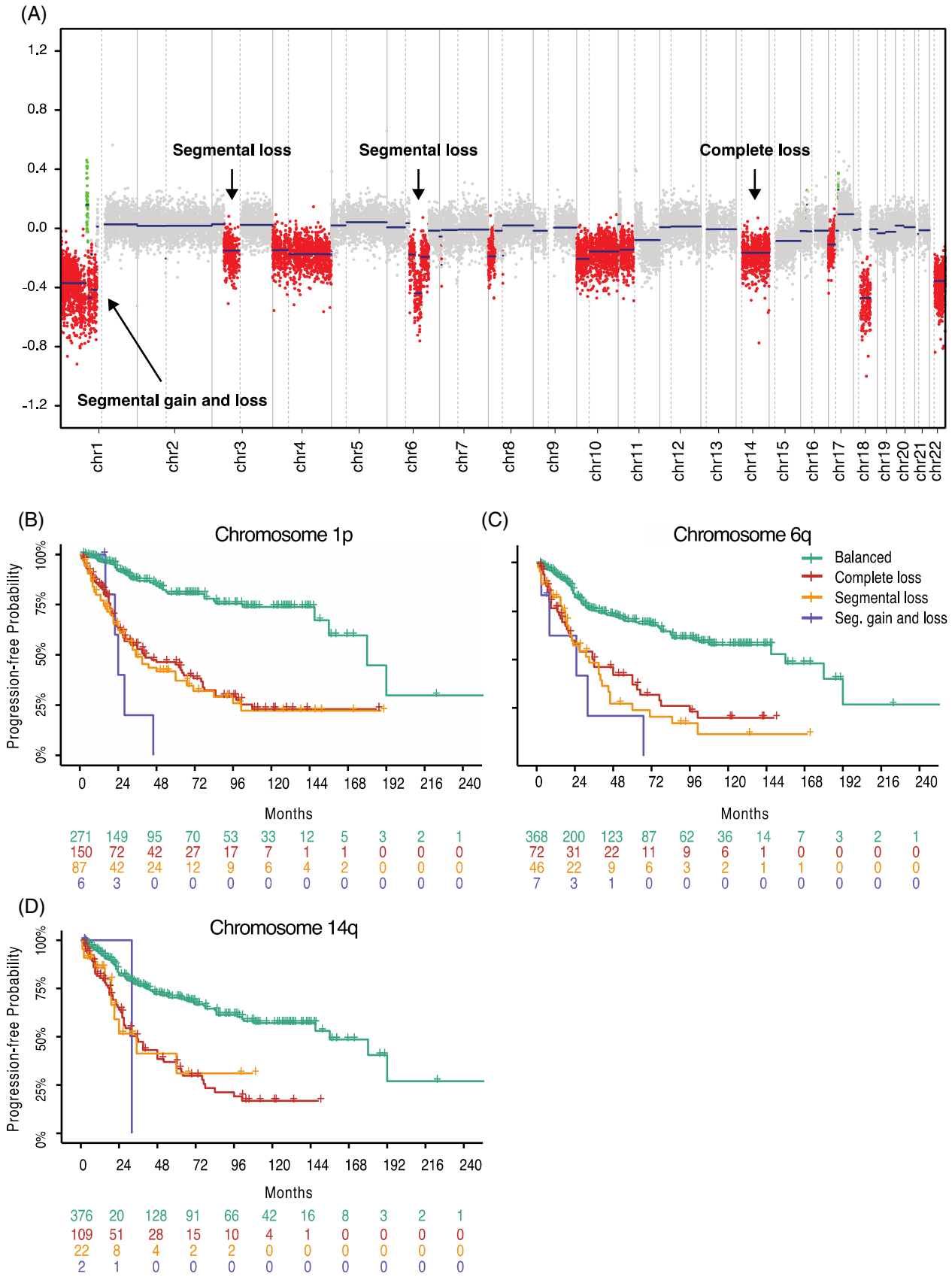

FIGURE 1 Any combination of a chromosomal arm that features a loss of at least 5% of the total arm is associated with increased risk for progression in 1p, 6q and 14q. An example CNV plot of a meningioma case shows the different options that features a chromosomal loss as defined by at least reduced signal in 5% or more of the chromosomal arm. This includes the complete loss of a chromosomal arm, a segmental loss between 5% and 95% of the chromosomal arm and a combination of a loss and a gain (A). Kaplan–Meier plots for chromosomal arms 1p, 6q and 14q of the different options that feature a loss indicate increased risk for progression when any loss is present and therefore the IntS awards points to any loss detected (B–D).

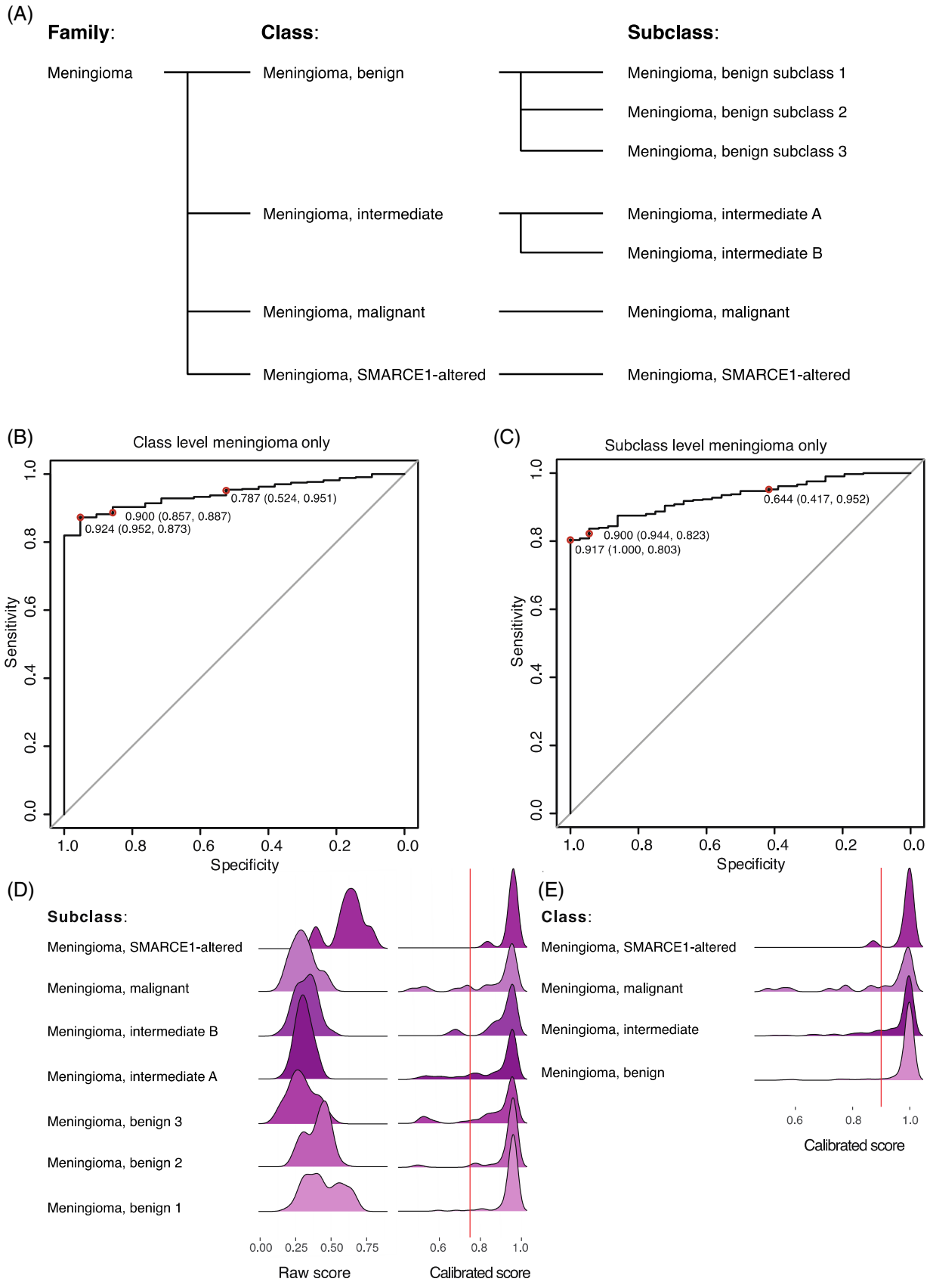

FIGURE 2 Legend on next page.

are comparable to the families identified in the meningioma classifier and thus include the benign, intermediate and malignant meningioma classes.

Additionally, meningiomas that harbor a *SMARCE1*-alteration have been shown to contain a very distinct methylation profile from other meningioma cases [15]. Histologically, *SMARCE1*-altered cases are most often clear cell meningioma that most often does not require further molecular risk assessment [7]. Likewise, the IntS is not compatible with cases identified to be part of the meningioma *SMARCE1*-altered methylation class. Classifier v12.5 methylation classes are then, if applicable, subdivided into methylation subclasses that include the previously identified ben-1, ben-2, ben-3, int-A and int-B groups (Figure 2A and Table S1). The v12.5 brain tumor classifier utilizes a bottom-up approach for the determination of calibrated scores. This means that, if applicable, the scores of subclasses are added together to determine the score of a class. Likewise, scores for classes are added together to determine the family score and thus the final score for the meningioma family is the accumulation of the scores attributed to the ben-1, ben-2, ben-3, int-A, int-B, mal and *SMARCE1*-altered meningioma subclasses.

To establish if the results from the v12.5 brain tumor classifier can be used as inputs for the intS, we first compared the prediction certainty obtained from the meningioma classifier v2.4 and the brain tumor classifier v12.5. To this end, all cases that were part of the discovery ($n = 514$), retrospective validation ($n = 184$) and prospective validation ($n = 287$) cohorts were analyzed using the v12.5 classifier. A total of 15 cases were excluded from further analysis as their v12.5 predicted outcome was either for the meningioma, *SMARCE1*-altered class ($n = 6$) or no matching score ($> = 0.9$) for the meningioma MF was obtained ($n = 9$) (Table S2). Results for the resulting 970 cases from the two classifiers were then compared. In the v2.4 classifier, 42.7% of cases have a calibrated family score below the 0.9 threshold. This number decreases to 12.3% with class score (note again the different terminology) below 0.9 in the v12.5 classifier. Similar results were obtained at the class (or defined in v12.5 as "subclass") level where the number of cases below 0.9 decreased from 51.8% to 23.3% (Table S3A–B). To validate the threshold level for the prediction of a v12.5 MF, class or subclass, a receiver operating characteristic curve analysis of the maximum calibrated scores was performed on either all available families, classes and subclasses or meningioma classes and subclasses only. For this, a binary class was defined based on the maximum calibrated score. Cases correctly called during cross-validation in the v12.5 reference set were determined "classifiable" and cases incorrectly classified were determined "non-classifiable". This analysis resulted in an optimal threshold of 0.954 for families, 0.966 for classes and 0.953 for subclasses. When only the meningioma classes and subclasses were included, the optimal threshold for the subclasses was 0.917–0.924 for the class (Figure 2B–C). Thresholds that achieve a minimum sensitivity of 0.95 are 0.87 for the class and 0.752 for the meningioma subclasses suggesting that in clinical practice a threshold of 0.9 for the family and class can be utilized and a threshold of 0.75 for the subclass. Plotting of the v12.5 subclass and class distribution shows that, especially at the class level, a small number of cases fall below the 0.9 cut-off implying an overall more confident prediction compared to the meningioma v2.4 classifier (Figure 2D–E).

We then investigated the stability of the methylation families when comparing the meningioma classifier v2.4 to the brain tumor classifier v12.5 by grouping cases into the highest scoring MF as is part of the standard IntS workflow as outlined above. In the meningioma classifier v2.4, the distribution of cases over the benign, intermediate and malignant families was 46.0%, 41.9% and 12.1% whereas this distribution shifted to 59.9%, 33.9% and 6.2% in the v12.5 brain tumor classifier indicating an, in general, "downgrading" of the MF (or class as these are now called in the v12.5 classifier). This was also observed when plotting the outcomes in a cross-over plot where cases were split at the 0.9 calibrated score threshold (Figure 3A). Here, the increase in cases that obtain a score of $> = 0.9$ is again identified, as well as the trend that if cases change in MF/class, this is most often a downgrading of the MF/class. A Kaplan–Meier (KM) plot of the two classifier outputs, shows a more aggressive course in patients identified to be part of the v12.5 intermediate and malignant meningioma classes (Figure 3B). Smaller changes in group crossover were observed when the IntS-based on inputs from the v12.5 brain tumor classifier over the v2.4 meningioma classifier were compared (Figure 3C). Distribution of cases over the low, intermediate and high groups was 43.7%/33.7%/22.6% for the v2.4 meningioma classifier input and 49.6%/30.1%/20.3% for the input obtained

FIGURE 2 The version 12.5 brain tumor classifier is calculated in a bottom-to-top manner by combining from subclasses to classes to families. The 12.5 version of the brain tumor classifier combines the previously established brain tumor classifiers and dedicated meningioma classifier. To account for the increased number of levels, previously identified meningioma families (benign, intermediate and/or malignant) are now called classes and the ben-1, ben-2, ben-3, int-a, int-B and mal classes are now defined as subclasses. Additionally, meningioma that harbor a *SMARCE1*-alteration are included as a separate class and subclass (A). Receiver operating characteristic (ROC) curve analysis of the maximum calibrated scores on meningioma classes (B) and subclasses only (C). For the ROC analysis, a binary class was defined based on the maximum calibrated score. Cases correctly called during cross-validation in the v12.5 reference set were determined "classifiable" and cases incorrectly classified were determined "non-classifiable". The v12.5 reference set includes 417 classifiable and 36 non-classifiable meningioma cases. Distribution of raw and calibrated scores for meningioma subclasses (D) and classes (E). The vertical lines indicated the proposed cut-off of 0.75 for subclasses and 0.9 for classes.

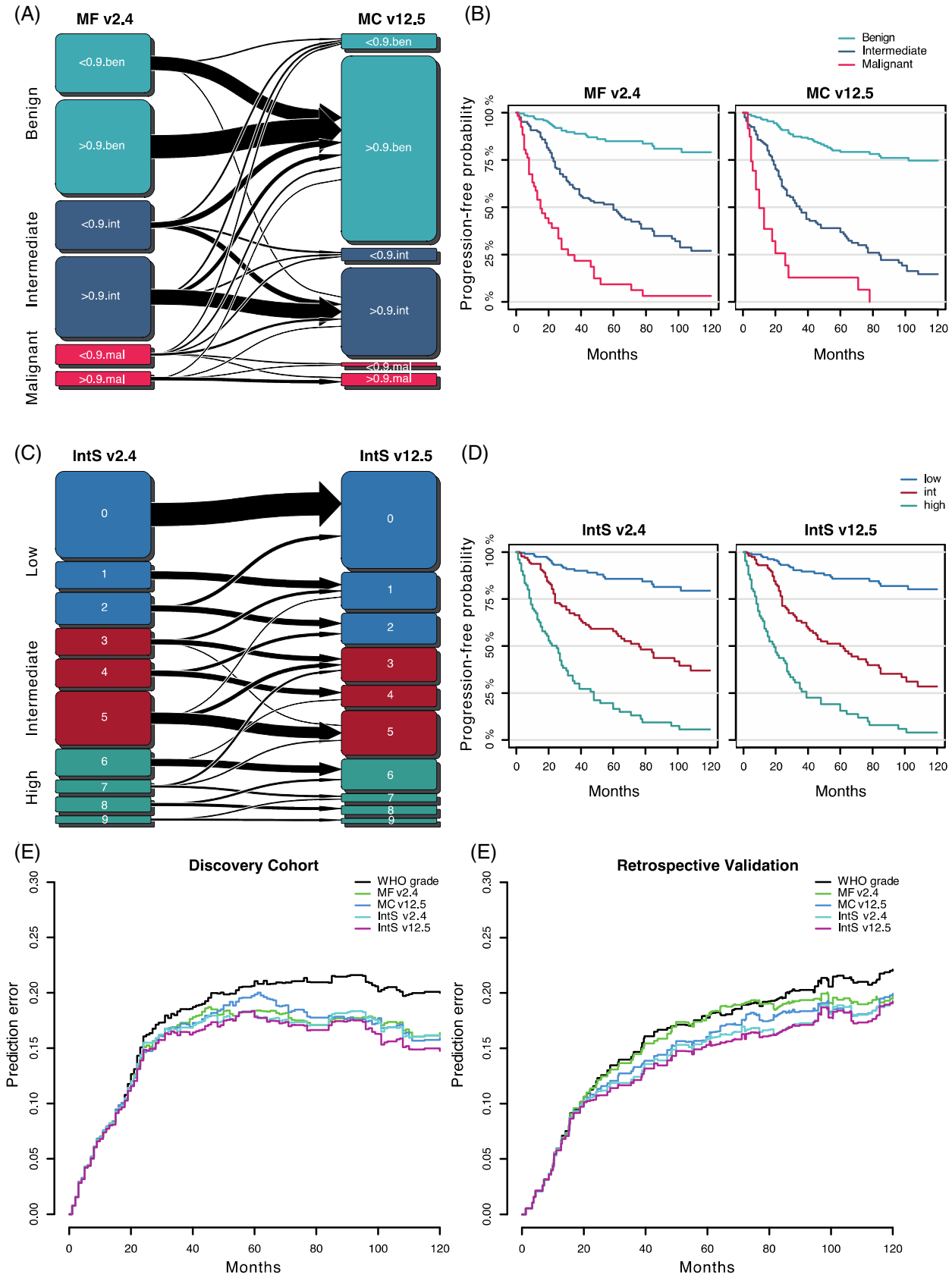

FIGURE 3 Legend on next page.

from the v12.5 brain tumor classifier. Accordingly, the KM-plots for the IntS-based on the input from the two classifiers are more similar and show a more equal distribution of risk over the three strata (Figure 3D). In the discovery cohort, 9.5% of cases moved to a different IntS risk prediction level. Specifically, out of the 232 patients identified as low risk in the IntS based on v2.4 input (IntS v2.4), 1 case was predicted to be at IntS intermediate risk when classifier v12.5 input (IntS v12.5) was used. In total, 174 cases were identified to be at intermediate IntS v2.4 risk, of which 31 cases moved to low risk and none to high risk in IntS v12.5. Out of the 100 IntS v2.4 high-risk patients, 15 moved to intermediate risk and 1 to low risk in IntS v12.5 risk prediction.

To determine the risk prediction accuracy, Brier score was plotted of risk prediction models based on histological WHO grading, methylation classifiers and the IntS risk determination based on inputs from the two different classifiers. This analysis was performed in the discovery, retrospective validation and prospective validation cohorts. In both the discovery and retrospective validation cohorts, the lowest error rate and thus highest prediction accuracy was obtained for the IntS based on MF inputs from the v12.5 brain tumor classifier (Figure 3E–F). IntS based on meningioma v2.4 classifier input outperformed the other methods in the prospective cohort (data not shown). More importantly though, the differences in integrated Brier score are very small (maximum of 0.004 at 5y and a maximum of 0.005 at 10y in all cohorts), with no clinical relevance (Tables 2 and 3). Similarly, although the difference in integrated Brier score in the discovery cohort was significant at 5y and 10y, these differences were non-significant in the retrospective cohort (Table 3). C-index was similar for the IntS based on inputs from the two classifiers (Tables 2 and S4) with non-significant differences between the two models of less than 1% that have no clinical relevance. It can therefore be concluded that, even though some individual cases may obtain a risk prediction that differs when input from the two different classifiers is used, on a population level no significant difference is observed in the prediction power for the IntS based on the two different classifiers. Simultaneously, our results reaffirmed the prediction power of the IntS as the IntS based on the v12.5 input performed better or outperformed the IntS based on meningioma v2.4 input when comparing with histological grading or MF prediction only (Tables 2, 3 and S4).

3.3 | Accuracy of a morphological WHO grade layer

The first layer of the IntS is based on the WHO grade and at the time of the IntS publication, the 2016 CNS WHO classification was the classification in use. As discussed before, mutations in the *TERT*-promoter and/or a homozygous loss of *CDKN2A*/*B* are newly added criteria to diagnose a grade 3 meningioma in the 2021 CNS5 classification. This suggests that the WHO grade layer in the IntS should perhaps include the *TERT*-promoter and *CDKN2A*/*B* status. This poses conceptual and practical challenges. First, the concept of the molecular-morphologic meningioma classification is that the individual layers attribute points to the morphologic-, epigenetic- and respective CNV-status. By including the two molecular markers into the WHO grade layer, this layer would include and require the investigation of specific molecular alterations and thus, to a certain extent, morph the different layers together. Second, although the *CDKN2A*/*B* status is known from the CNV plot when a genome-wide methylation array is performed, the investigation of the *TERT*-promoter status would require an additional molecular test and thus increase the costs.

To investigate to what extent *TERT*-promoter mutations and homozygous losses of *CDKN2A*/*B* are observed in the different IntS strata, we extracted this data from the discovery cohort as this cohort is the only cohort with sufficient data on *TERT*-promoter mutations available (Table S5). Data on the 2021 CNS5 WHO grading status was available for 389 cases (enriched for histological grade 2 and 3), of which 24 cases had a *TERT*-promoter mutation. When only the meningioma classifier v2.4 MF or the v12.5 brain tumor classifier class was used to stratify for progression risk, 10 and 14, respectively, of these 24 *TERT*-mutant cases were annotated as benign or intermediate risk with both classifiers scoring one case as benign. In the IntS, regardless of WHO grading scheme, no cases annotated as low risk contain a *TERT*-promoter mutation. When the 2016 morphological grading is used, 2 cases were predicted to have intermediate risk in the v2.4 meningioma classifier and 3 cases with the v12.5

FIGURE 3 Class prediction accuracy improves in the new v12.5 brain tumor classifier compared to the dedicated meningioma classifier with no loss in prognostic power of the IntS. Cross-over plot showing the obtained scores for methylation families/classes as obtained from the dedicated meningioma classifier v2.4 or the new brain tumor classifier v12.5 shows an increase in the percentage of cases that obtains a calibrated score of $>= 0.9$ (A). Fewer cases are aliquoted to the intermediate and malignant families/classes in the v12.5 classifier compared with the v2.4 classifier. Intermediate and malignant cases in v12.5 show a more aggressive course compared to v2.4 (discovery cohort $n = 514$ shown) (B). A smaller number of group cross-overs is observed when comparing the IntS based on input from v2.4 or v12.5 (C). Likewise, smaller differences in the Kaplan–Meier plots are detected (discovery cohort $n = 514$ shown) (D). Brier prediction score analysis shows lower error rate, thus higher prediction accuracy the IntS based on inputs from the v12.5 classifier compared to the v2.4 classifier. These differences are only significant in the discovery cohort and even more importantly are very small (maximum of 0.004 at 5y and a maximum of 0.005 at 10y in all cohorts) implying that in daily practice using the input from the v2.4 meningioma classifier or the v12.5 brain tumor classifier does not make a difference (E).

TABLE 2 Integrated brier score (IBS) and c-index for the different risk prediction models in the discovery, retrospective and prospective cohorts

	Riskfactor	Distribution	c-index	IBS 5y	IBS 10y
Discovery cohort	WHO grade	46.2/41.7/12.1	0.699	0.143	0.175
	MF v2.4	48.2/40.9/10.9	0.728	0.134	0.154
	MC v12.5	63.2/31.6/5.1	0.727	0.135	0.155
	IntS v2.4	45.8/34.4/19.8	0.751	0.132	0.152
	IntS v12.5	52.0/31.4/16.6	0.761	0.129	0.147
Retrospective validation	WHO grade	58.0/35.4/6.6	0.665	0.117	0.159
	MF v2.4	45.3/45.9/8.8	0.678	0.115	0.153
	MC v12.5	59.7/34.3/6.1	0.707	0.106	0.143
	IntS v2.4	47.5/33.1/19.3	0.727	0.104	0.139
	IntS v12.5	51.4/32.0/16.6	0.735	0.101	0.135
Prospective validation	WHO grade	66.8/33.2/0.0	0.600	0.080	
	MF v2.4	68.9/29.3/1.8	0.596	0.073	
	MC v12.5	83.0/16.3/0.7	0.577	0.071	
	IntS v2.4	69.3/25.1/5.7	0.666	0.076	
	IntS v12.5	73.5/22.6/3.9	0.622	0.072	

TABLE 3 Integrated brier score (IBS) score p-value comparison between the methylation based predictive models in the discovery, retrospective and prospective cohorts

	IBS 5y discovery	IBS 5y retrospective validation	IBS 10y discovery	IBS 10y retrospective validation
WHO 2016 versus MF v2.4	0.0160	0.8645	0.0010	0.4684
WHO 2016 versus MC v12.5	0.0027	0.0413	<0.001	0.0520
WHO 2016 versus IntS v2.4	<0.001	0.0293	<0.001	0.0068
WHO 2016 versus IntS v12.5	<0.001	0.0061	<0.001	0.0015
MF v2.4 versus MC v12.5	0.1848	0.4070	0.2650	0.3798
MF v2.4 versus IntS v2.4	0.0297	0.0254	0.1289	0.0816
MF v2.4 versus IntS v12.5	0.0199	0.0342	0.0457	0.0890
MC v12.5 versus IntS v2.4	0.0528	0.2986	0.0689	0.4144
MC v12.5 versus IntS v12.5	0.0038	0.0733	0.0076	0.1049
IntS v2.4 versus IntS v12.5	0.0492	0.8551	0.0201	0.6077

brain tumor classifier. For both classifiers, the IntS based on the 2021 CNS5 WHO grading only has a single case annotated as intermediate risk. This is due to the increased number of points awarded for the WHO grading layer as a *TERT*-promoter mutation would automatically award two points for this layer.

All 506 discovery cohort cases that remained after filtering had information on the *CDKN2A*/*B* status, of which the 389 sequenced cases had a fully compatible CNS5 WHO grade available (Table S6). Similar results to the *TERT*-promoter analysis were observed for the *CDKN2A*/*B* status with also a single intermediate-risk patient in the 2021 CNS5 WHO grading-based IntS model. Taken together, these data on *TERT*-promoter and *CDKN2A*/*B* homozygous loss data suggest that the IntS based on the 2021 CNS5 WHO grading may be the most accurate in risk prediction, but an IntS based on morphological 2016 WHO Grading without assessment of *TERT*-promoter and *CDKN2A*/*B* status also almost exclusively attributes cases with such a molecular alteration to the highest risk group. The inclusion of an additional molecular test (i.e., *TERT*-promotor, *CDKN2A*/*B*) in the histology layer will therefore most likely have limited additional value for risk prediction in meningiomas.

4 | DISCUSSION

Prior to the 2021 CNS5 WHO classification, grading (and thus risk prediction) was solely based on morphological features. Although high-risk meningiomas are most often identified by high mitotic activity, pleomorphism and necrosis, accurate discrimination between low and intermediate-risk meningiomas can be challenging, when based solely on morphological features. Both the mitotic count and the combination of specific

morphological features (i.e., increased cellularity, high nucleus to cytoplasm ratio, prominent nucleoli, sheeting and focal necrosis) are subject to high interobserver variation [16]. To address these inconsistencies, multiple approaches to incorporate information regarding the molecular underlying of a meningioma tumor, have been suggested in today's literature. These improved grading models range from the inclusion of specific mutations, CNVs, epigenetic status of the tumor cells or a combination of these markers as we did in 2021 with the publication of the molecular-morphologic IntS. With the inclusion of the *TERT*-promoter mutation and homozygous loss of *CDNK2A*/*B* as criteria for grade 3 meningioma, specific individual molecular alterations have been included in the official WHO criteria whereas more advanced and integrated models remain promising yet optional methods for risk prediction in meningioma. A reason for the lack of adoption into official grading methods could be the lack of information regarding the practical implementation of the prediction model, or no further "support" after the publication of the model when new information or novel methods for the implementation becomes available. In this manuscript, we provide further information on the clinical implementation of the IntS and investigated the applicability of the new 12.5 version of the brain tumor classifier.

Epigenetic whole genome meningioma multivariable risk prediction started with the 2017 publication that divided meningioma in benign, intermediate and malignant methylation families that were associated with low, intermediate and high risk for progression respectively [5]. As discussed above, the benign and intermediate families are then further separated in classes that have approximately the same progression risk, yet have different epigenetic features. Although highly informative, a prediction model purely based on the epigenetic features ignores other morphological, molecular or clinical information. A later study presented a risk prediction model based on the epigenetic risk, combined with the WHO grade and Simpson grade [17]. The same group later published an even more extensive model, where four meningioma risk groups were identified based on data from methylation-arrays, RNA sequencing and CNV data [18]. Although this complete model is not readily translatable to clinical practice, four immunohistochemical stainings were proposed as proxy markers of the four molecular risk groups possibly reducing implementation costs significantly [18]. Additionally, analysis of the in vitro and in vivo experiments suggested that the four risk groups have different biological traits and are thus susceptible to different treatments. The same conclusion was reached by a different study that identified three epigenetic-based risk groups that were either Merlin-intact (the protein coded by the *NF2* gene), immune-enriched or hypermitotic [19]. With the data from this study, clinical risk prediction for an individual patient can be further pinpointed by inclusion of the WHO-grade, MIB1 labeling percentage, recurrent status of the tumor, extent of the surgical resection, patient sex and whether or not the patient received adjuvant radiotherapy [19]. To avoid the need for a specific whole genome methylation array, one recently published integrated risk model is based on specific CNVs of chromosomal arms or *CDKN2A*/*B* specifically, combined with the mitotic count to separate three risk levels [6]. The information needed for this model can be obtained from different techniques including nanopore sequencing that may require fewer investments to obtain [20]. This is in line with the fact that access to a methylation array was the biggest drawback to the worldwide implementation of the integrated risk models discussed above. With the publication of the 2021 CNS5 WHO classification though, access to methylation-array-based methods should be within the toolbox of every neuropathology department as specific diagnoses such as "high-grade astrocytoma with piloid features" require the utilization of such methods to make an accurate diagnosis [2, 21]. This should increase the availability of this technique and in parallel the adoption of the different risk prediction models and could lead to independent validation and comparison of the proposed models. This will also require the authors proposing these models to adapt to technical questions, challenges and changes underlying their model.

In daily practice, appropriately attributing resources regarding molecular testing remains a challenge. In this manuscript, we propose that when a methylation-array is utilized to determine the IntS, additional testing for a *TERT*-promoter mutation may not be needed. Similarly, the methylation-array provides the *CDNK2A*/*B* status, but including the *TERT*-promoter and *CDNK2A*/*B* CNV data into the histology/WHO grading layer of the IntS does not provide much additional predictive accuracy. To save resources and costs we thus propose to exclude both markers from the IntS. In situations where a methylation-array may not be feasible, specific testing for these alterations may however still be the most cost-efficient way to include molecular data in the risk prediction process of meningiomas as proposed by the 2021 CNS5 WHO classification.

5 | CONCLUSIONS

With this manuscript, we illustrated that chromosomal losses of over 5% of the chromosomal arm can be regarded as a loss in our prediction models. Additionally, we show that incorporation of the information from the new brain tumor classifier v12.5, which includes both the identification of the meningioma MF as well as the subsequent meningioma classes and subclasses, is similar or better for the establishment of the IntS than the information obtained from the dedicated meningioma classifier v2.4. Finally, we show that there is most likely no need

to include information regarding the *TERT*-promoter and/or *CDKN2A*/*B* homozygous loss status, in the WHO grade layer of the IntS, and that thus a purely histological WHO grade suffices for accurate risk prediction using the IntS. This information should help neuropathologists and other clinicians involved with the care for meningioma patients to incorporate the IntS for risk prediction and help select and stratify patients for meningioma trials, as well as in the consideration regarding the need for adjuvant therapies for their patients suffering from meningioma.

AUTHOR CONTRIBUTIONS

Thomas Hielscher, Felix Sahm and Sybren L.N. Maas designed the study. Thomas Hielscher and Martin Sill performed the data analysis. Study material was supplied by Martin Sill, Philipp Sievers, Damian Stichel, Sebastian Brandner, David T.W. Jones and Andreas von Deimling. All authors were involved in the interpretation and clinical translation of the data. All authors were involved in the writing of the manuscript.

ACKNOWLEDGMENT

Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

This study was supported by the German Cancer Aid (Grant No. 70112956), and the Hertie Foundation (Hertie Network of Excellence in Clinical Neuroscience).

CONFLICT OF INTEREST

The authors declare no specific conflicts of interest regarding this study.

DATA AVAILABILITY STATEMENT

Upon reasonable request, the DNA methylation and clinical outcome data can be shared.

ETHICS STATEMENT

All clinical data included in this manuscript was previously published and collected with approval according to local regulators supplemented with patient consent from collaborating centers in Germany, Switzerland, United States, United Kingdom and Austria.

ORCID

Sebastian Brandner https://orcid.org/0000-0002-9821-0342

Sybren L. N. Maas https://orcid.org/0000-0002-8745-4405

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hielscher T, Sill M, Sievers P, Stichel D, Brandner S, Jones DTW, et al. Clinical implementation of integrated molecular-morphologic risk prediction for meningioma. Brain Pathology. 2022. e13132. https://doi.org/10.1111/bpa.13132