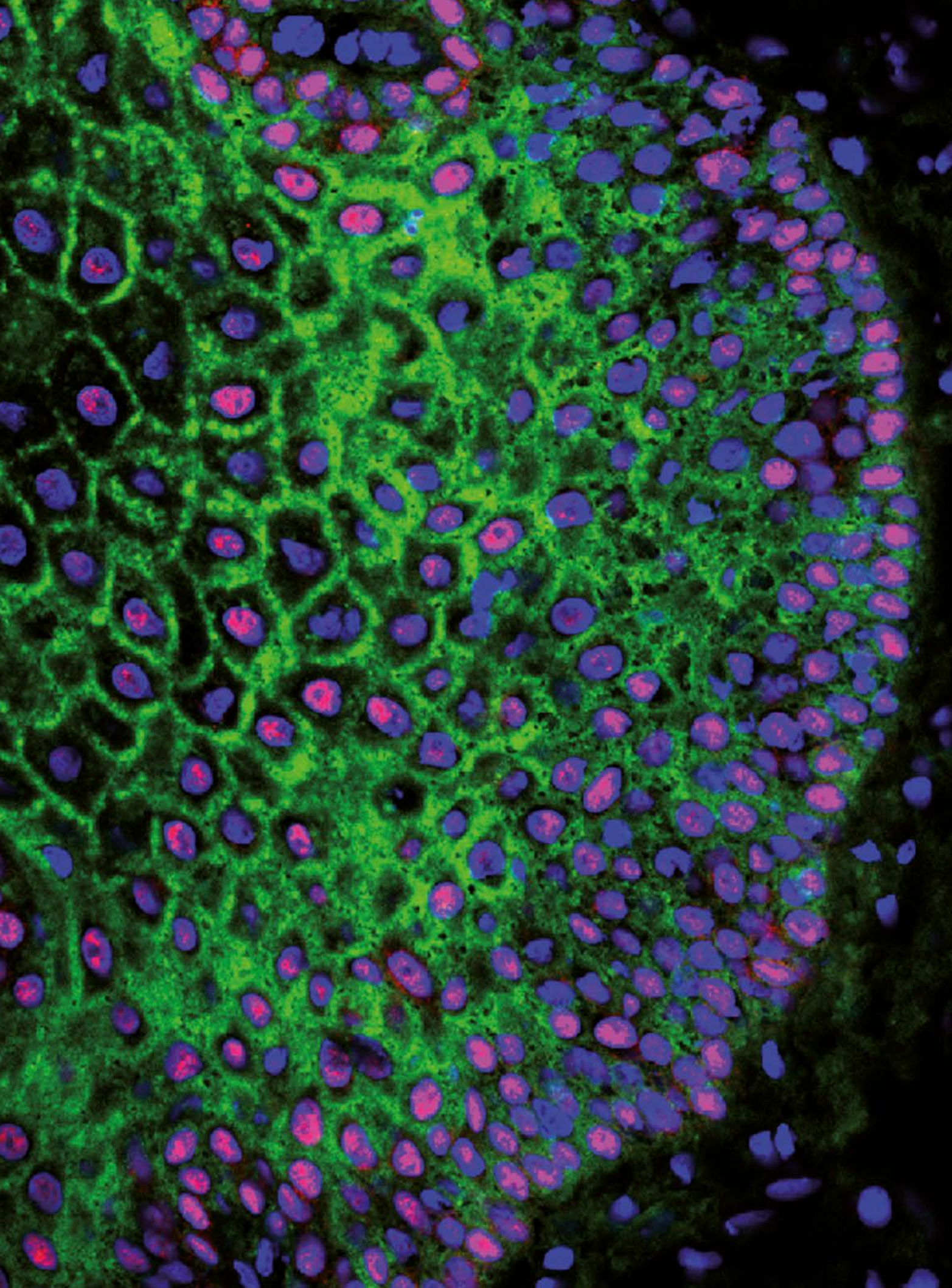

Section of Molecular Pathology (MPA)

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The Section of Molecular Pathology (MPA) conducts original research to elucidate the molecular basis and genetic pathways of human neoplasms. MPA's specific aims are to provide genetic information that will be used as the basis for future molecular diagnosis and classification of brain tumours, to identify genetic markers for prognosis and novel treatment strategies, and to use genetic data to identify new clues to understand the etiology of human tumours (Kim et al., 2014a; Louis et al., 2014; Ohgaki et al., 2014). Genetic studies are carried out, using tumour samples from patients with excellent clinical data that have been collected at a population level or internationally, to provide unique data combining the pathology, genetics, clinical features, and epidemiology of tumours. MPA's research programme is a key element of IARC's goals of elucidating the mechanisms of carcinogenesis and understanding the etiology of cancer.

MPA is also responsible for the World Health Organization (WHO) Classification of Tumours series (WHO Blue Books). MPA works with internationally recognized pathologists from around the world to reach consensus regarding tumour classification. Most human tumours have been diagnosed and classified based on histological features; more recently, molecular markers are increasingly being used to

define disease entities, taking advantage of rapid progress in the understanding of the genetics of human neoplasms.

Several of the main projects of MPA over the 2014–2015 biennium are detailed below.

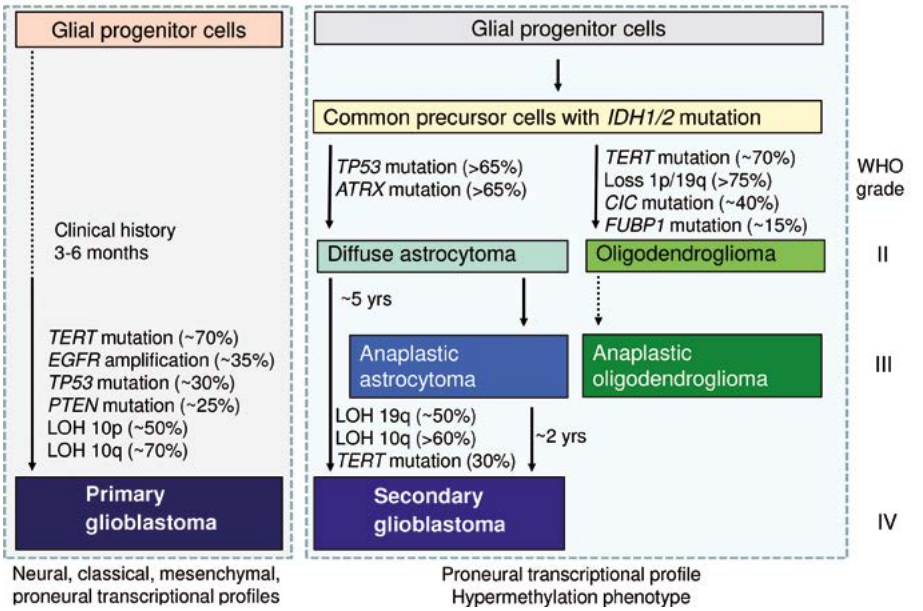
Alterations of the *RRAS* and *ERCC1* genes at 19q13 in gemistocytic astrocytomas

Gemistocytic astrocytoma (WHO grade II) is a rare variant of diffuse astrocytoma, characterized by the presence of neoplastic gemistocytes and a consistent tendency to progress to secondary glioblastoma (WHO grade IV) and have a poor prognosis. Other than frequent *TP53* mutations (> 80%), little has been known about the molecular profile of gemistocytic astrocytomas. Exome sequencing was carried out in gemistocytic astrocytomas, and homozygous deletion of genes was identified at 19q13, i.e. *RRAS* and *ERCC1*. Further screening showed *RRAS* homozygous deletion in 7 of 42 (17%) gemistocytic astrocytomas and in 3 of 24 (13%) secondary glioblastomas. Patients with gemistocytic astrocytoma and secondary glioblastoma with *RRAS* deletion tended to have shorter survival times than those without deletion. Also, *ERCC1* homozygous deletion or promoter methylation was found in 10 of 42 (24%) gemistocytic astrocytomas and in 8 of 24 (33%) secondary glioblastomas. Homozygous deletions of *RRAS* and *ERCC1* were absent in other low-grade diffuse gliomas and in primary (de novo) glioblastomas (Ohta et al., 2014).

Loss of FUBP1 expression in gliomas predicts *FUBP1* mutation and is associated with oligodendroglial differentiation, *IDH1* mutation, and 1p/19q loss

Far upstream element-binding protein 1 (FUBP1) regulates several target genes, such as *MYC* and *p21*. FUBP1 is upregulated in a variety of tumours and acts as an oncoprotein by stimulating proliferation and inhibiting apoptosis. FUBP1 expression profiles in gliomas were examined by immunohistochemistry and immunofluorescence. FUBP1 expression was higher in all glioma subtypes compared with normal brain tissue, and was associated with increased cell proliferation. Loss of FUBP1 expression predicted *FUBP1* mutation with a sensitivity of 100% and a specificity of 90%, and was associated with oligodendroglial differentiation, *IDH1* mutation, and 1p/19q loss, suggesting that FUBP1 immunohistochemistry is useful for glioma diagnosis (Baumgarten et al., 2014).

Figure 1. Genetic pathways to primary and secondary glioblastomas. Note that secondary glioblastomas share a common origin of cells with oligodendrogliomas. © IARC.



The Olig2 labelling index is correlated with histological and molecular classifications in low-grade diffuse gliomas

Diagnosis of low-grade diffuse gliomas based on histology is highly subjective, with significant inter-observer variability. Olig2 expression was assessed by immunohistochemistry in WHO grade II diffuse astrocytomas, oligoastrocytomas, and oligodendrogliomas. The mean Olig2 labelling index was 44% in diffuse astrocytomas, 59% in oligoastrocytomas, and 76% in oligodendrogliomas. The Olig2 labelling index was significantly higher in gliomas with 1p/19q loss with or without *IDH1/2* mutation than in those carrying *TP53* mutation with or without *IDH1/2* mutation or in those with *IDH1/2* mutation only (Suzuki et al., 2014).

TP53, *MSH4*, and *LATS1* germline mutations in a family with clustering of nervous system tumours

Exome DNA sequencing of blood samples from a Li–Fraumeni family with a *TP53* germline mutation and multiple nervous system tumours revealed additional germline mutations. Missense mutations in the *MSH4* DNA repair gene (c.2480T > A; p.I827N) were detected in three patients with gliomas. Two family members without a *TP53* germline mutation who developed peripheral schwannomas also carried the *MSH4* germline mutation and, in addition, a germline mutation of the *LATS1* gene (c.286C > T; p.R96W). LATS1 is a downstream mediator of NF2 but has not previously been found to be related to schwannomas. Therefore, the entire coding sequence of the *LATS1* gene in sporadic schwannomas was screened, and a single base deletion at codon 827 was found in a spinal schwannoma. Mutational loss of LATS1 function may thus play a role in some inherited schwannomas, but only exceptionally in sporadic schwannomas. This is the first study reporting a germline *MSH4* mutation. Since it was present in all patients, it may have contributed to the subsequent acquisition of *TP53* and *LATS1* germline mutations (Kim et al., 2014a).

Alterations in the NF2/LATS1/LATS2/YAP pathway in schwannomas

Schwannoma is a benign nerve sheath tumour composed of well-differentiated Schwann cells. Other than frequent *NF2* mutations (50–60%), the molecular basis of schwannomas is not fully understood. LATS1 and LATS2 are downstream molecules of NF2 and negative regulators of the *YAP* oncogene in the Hippo signalling pathway. MPA assessed mutations of the *NF2*, *LATS1*, and *LATS2* genes, promoter methylation of *LATS1* and *LATS2*, and expression of YAP and phosphorylated YAP (pYAP) in 82 sporadic schwannomas. Targeted sequencing using the Ion Torrent Proton instrument revealed *NF2* mutations in 45 (55%) schwannomas, *LATS1* mutations in 2 (2%) schwannomas, and *LATS2* mutations in 1 (1%) schwannoma. Methylation-specific polymerase chain reaction (PCR) showed promoter methylation of *LATS1* and *LATS2* in 14 (17%) and 25 (30%) cases, respectively. Overall, 62 (76%) cases had at least one alteration in the *NF2*, *LATS1*, and/or *LATS2* genes. Immunohistochemistry revealed nuclear YAP expression in 18 of 42 (43%) and reduced cytoplasmic pYAP expression in 15 of 49 (31%) schwannomas analysed, all of which had at least one alteration in the *NF2*, *LATS1*, and/or *LATS2* genes. These results suggest that an abnormal Hippo signalling pathway is involved in the pathogenesis of the majority of sporadic schwannomas (Oh et al., 2015).

Role of microRNAs in the pathogenesis and progression of medulloblastomas

Medulloblastoma is the most frequent malignant central nervous system tumour in children. MicroRNAs (miRs) are small, non-coding RNAs that target protein-coding and non-coding RNAs and play roles in a variety of cellular processes through regulation of multiple targets.

MPA assessed miR-22 expression and its effect on cell proliferation and apoptosis in medulloblastomas. Quantitative reverse transcription PCR (RT-PCR) revealed significantly lower expression of miR-22 in 19 of 27 (70%) medulloblastomas and three medulloblastoma cell lines, compared with normal cerebellum. Forced expression of miR-22 by lentiviral vector transfection reduced cell proliferation and induced apoptosis, while knockdown of miR-22 increased proliferative activity in DAOY and ONS-76 medulloblastoma cells. Microarray analysis in DAOY cells with forced miR-22 expression showed significant changes in expression profiles; *PAPST1* was the most significantly (10-fold) downregulated gene. Quantitative RT-PCR revealed *PAPST1* mRNA upregulation in 18 of 27 (67%) medulloblastomas. In addition, a luciferase reporter assay suggested that miR-22 directly targets the *PAPST1* gene, and lentivirus-mediated knockdown of *PAPST1* suppressed proliferation of medulloblastoma cells. These results suggest that frequently downregulated miR-22 expression is associated with cell proliferation in medulloblastomas, and this may be at least in part via *PAPST1*, which is a novel target of miR-22 (Xu et al., 2014).

miR-9, a key regulator of neuronal development, is aberrantly expressed in brain malignancies. MPA showed that miR-9 expression is frequently downregulated in medulloblastomas, and that this is at least in part due to promoter methylation. Low miR-9 expression correlated significantly with the diagnosis of unfavourable histopathological variants and with poor clinical outcome. Furthermore, *HES1* was identified as a direct target of miR-9 in medulloblastoma. Restoration of miR-9 was shown to trigger cell cycle arrest, inhibit clonal growth, and promote cell differentiation. Re-expression of miR-9 may constitute a novel epigenetic regulation strategy against medulloblastomas (Fiaschetti et al., 2014).

WHO Classification of Tumours series (WHO Blue Books)

The objective of this project is to establish a histopathological and molecular classification and grading of human tumours that is accepted and used worldwide. Without clearly defined clinical and histopathological diagnostic criteria and, more recently, genetic and expression profiles, epidemiological studies and clinical trials are difficult to conduct. Therefore, this project is of great importance not only for pathology communities but also for cancer registration, epidemiological studies, clinical trials, and cancer research in general.

IARC has been responsible for this project since the third edition (2000–2005; 10 volumes). The current (fourth) edition of the WHO Classification of Tumours series was initiated in 2006 with four new series editors (Dr Fred Bosman, University of Lausanne, Switzerland; Dr Elaine Jaffe, National Institutes of Health, Bethesda, USA; Dr Sunil Lakhani, University of Queensland, Brisbane, Australia; and Dr Hiroko Ohgaki, IARC). So far, seven volumes have been published, and for each volume, 20 000–50 000 copies were printed and distributed worldwide.

In 2014–2015, the sixth volume (Tumours of Female Reproductive Organs) and seventh volume (Tumours of the Lung, Pleura, Thymus and Heart) have been published, and preparation of the eighth volume (Tumours of the Urinary System and Male Genital Organs), the ninth volume (Head and Neck Tumours), and the tenth volume (Tumours of Endocrine Organs) is under way. In addition, updates to the first and second volumes of the fourth edition, Tumours of the Central Nervous System and Tumours of Haematopoietic and Lymphoid Tissues, are in progress.

Figure 2. Cover of *WHO Classification of Tumours of Female Reproductive Organs*, fourth edition.

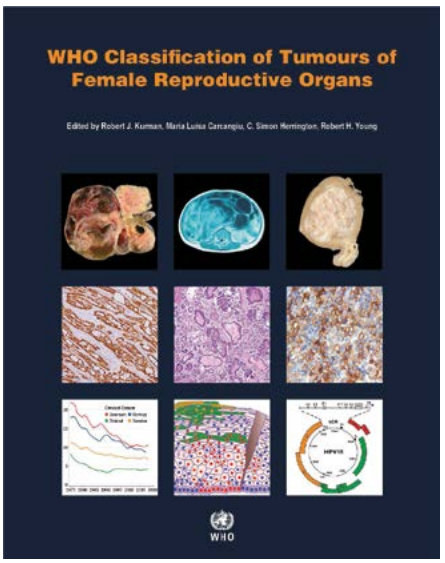


Figure 3. Working Group members at the consensus and editorial meeting of *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart.* The meeting was held at IARC on 24–26 April 2014. © IARC/Roland Dray.

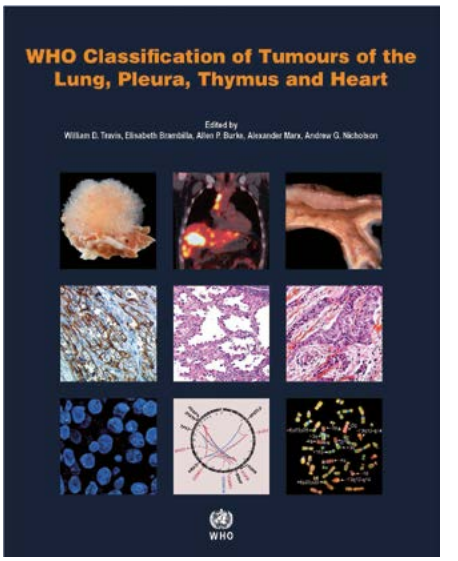
The sixth volume, Tumours of Female Reproductive Organs, was published in April 2014. It was edited by four volume editors (Dr Robert J. Kurman, Johns Hopkins University, Baltimore, USA; Dr Maria Luisa Carcangiu, Fondazione IRCCS, Institue Nazionale dei Tumori, Milan, Italy; Dr C. Simon Herrington, Centre for Oncology and Molecular Medicine, Ninewells Hospital and Medical School, Dundee, United Kingdom; and Dr Robert H. Young, Massachusetts General Hospital, Harvard Medical School, Boston, USA) and prepared by 92 authors from 18 countries.

The seventh volume, Tumours of the Lung, Pleura, Thymus and Heart, was published in March 2015. It was edited by five volume editors (Dr William D. Travis, Memorial Sloan Kettering Cancer Center, New York, USA; Dr Elisabeth Brambilla, Centre Hospitalier Universitaire de Grenoble, France; Dr Allen P. Burke, University of Maryland, Baltimore, USA; Dr Alexander Marx, University Medical Centre Mannheim, University of Heidelberg, Mannheim, Germany; and Dr Andrew G. Nicholson, Royal Brompton Hospital, London, United Kingdom) and prepared by 157 authors from 29 countries.

The eighth volume, Tumours of the Urinary System and Male Genital Organs, is being edited by four volume editors (Dr Holger Moch, University Hospital Zurich, Zurich, Switzerland; Dr Peter A. Humphrey, Yale University School of Medicine, New Haven, USA; Dr Thomas M. Ulbright, IU Health Pathology Laboratory, Indiana University School of Medicine, Indianapolis, USA; and Dr Victor E. Reuter, Memorial Sloan Kettering Cancer Center, New York, USA) and prepared by 103 authors from 19 countries. The consensus and editorial meeting was held in collaboration with the University of Zurich on 11–13 March 2015, and the book is scheduled to be published in early 2016.

The ninth volume, Head and Neck Tumours, is being prepared by five volume editors (Dr Adel K. El-Naggar, MD Anderson Cancer Center, Houston, USA; Dr John K.C. Chan, Queen Elizabeth Hospital, Hong Kong Special Administrative Region, China; Dr Jennifer R. Grandis, Clinical and Translational Science Institute, UCSF School of Medicine, San Francisco, USA; Dr Takashi Takata, Hiroshima University, Hiroshima, Japan; and Dr Pieter J. Slootweg, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands). The consensus and editorial meeting is scheduled for January 2016.

Figure 4. Cover of *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart,* fourth edition.



MPA is grateful to the following for their collaboration:

S.R. Lakhani, Brisbane, K. McDonald, Sydney, Australia; S.Q. Lv, Chongqing, J.K.C. Chan, Hong Kong Special Administrative Region, China; A. Vital, Bordeaux, E. Brambilla, Grenoble, J. Lachuer, Lyon, D. Figarella-Branger, Marseille, France; H. Stein, Berlin, Jürgen Thiele, Cologne, G. Reifenberger, Düsseldorf, U. Sure, Essen, M. Mittelbronn, Frankfurt, A. von Deimling, O.D. Wiestler, Heidelberg, R. Siebert, Kiel, A. Marx, Mannheim, G. Kloppel, Munich, W. Paulus, Münster, Germany; S.A. Pileri, Bologna, F. Giangaspero, Rome, Italy; Y. Nakazato, Gunma, T. Takata, Hiroshima, H. Yokoo, Maebashi, Japan; P.J. Slootweg, Nijmegen, The Netherlands; E. Campo, Barcelona, Spain; F. Bosman, Lausanne, M.A. Grotzer, P. Kleihues, H. Moch, T. Shalaby, M. Weller, Zurich, Switzerland; S. Herrington, Dundee, A. Nicholson, London, United Kingdom; A. Burke, R. Kurman, Baltimore, E.S. Jaffe, Bethesda, R.P. Hasserjian, N.L. Harris, D.N. Louis, R. Young, Boston, M.M. Le Beau, Chicago, A.K. El-Naggar, Houston, T.M. Ulbright, Indianapolis, D.W. Ellison, Memphis, P.A. Humphrey, New Haven, A. Orazi, V.E. Reuter, W.D. Travis, New York, J.R. Grandis, S.H. Swerdlow, Pittsburgh, W.K. Cavenee, San Diego, A. Perry, San Francisco, D.A. Arber, Stanford, USA.

Financial support from the following bodies is gratefully acknowledged:

Charles Rodolphe Brupbacher Foundation, Switzerland
Krebsliga Zürich, Switzerland
MEDIC Foundation, Switzerland