Methods in Pathology

Towards an international pediatric liver tumor consensus classification: proceedings of the Los Angeles COG liver tumors symposium

Dolores López-Terrada¹, Rita Alaggio², Maria T de Dávila³, Piotr Czauderna⁴, Eiso Hiyama⁵, Howard Katzenstein⁶, Ivo Leuschner⁷, Marcio Malogolowkin⁸, Rebecka Meyers⁹, Sarangarajan Ranganathan¹⁰, Yukichi Tanaka¹¹, Gail Tomlinson¹², Monique Fabrè¹³, Arthur Zimmermann¹⁴ and Milton J Finegold¹

¹Department of Pathology, Texas Children's Hospital, Baylor College of Medicine, Houston, TX, USA;

²Division of Pathology, Department of Medicine-DIMED, Pathology Unit, Padova, Italy; ³Departamento de Patologia, Hospital de Pediatría Prof. Dr. J.P. Garrahan, Buenos Aires, Argentina; ⁴Department of Surgery and Urology for Children and Adolescents, Medical University of Gdansk, Gdansk, Poland; ⁵Department of Surgery, Natural Science Center for Basic Research and Development, Hiroshima University Hospital, Hiroshima, Japan; ⁶Aflac Cancer Center, Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, GA, USA; ⁷Institut für Pathologie, UNI-Klinikum Campus, Kiel, Germany; ⁸Department of Pediatric Oncology, Medical College of Wisconsin, Milwaukee, WI, USA; ⁹Department of Pediatric Surgery, Primary Children's Medical Center, University of Utah, Salt Lake City, UT, USA; ¹⁰Department of Pathology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, USA; ¹¹Division of Pathology, Kanagawa Children's Medical Center, Yokohama, Japan; ¹²Division of Pediatric Hematology-Oncology, University of Texas Health Science Center, San Antonio, TX, USA; ¹³Department of Pathology, Institut de Cancerologie Gustave Roussy, Villejuif, France and ¹⁴Institute of Pathology, University of Bern, Bern, Switzerland

Liver tumors are rare in children, and their diagnoses may be challenging particularly because of the lack of a current consensus classification system. Systematic central histopathological review of these tumors performed as part of the pediatric collaborative therapeutic protocols has allowed the identification of histologic subtypes with distinct clinical associations. As a result, histopathology has been incorporated within the Children's Oncology Group (COG) protocols, and only in the United States, as a risk-stratification parameter and for patient management. Therefore, the COG Liver Tumor Committee sponsored an International Pathology Symposium in March 2011 to discuss the histopathology and classification of pediatric liver tumors, and hepatoblastoma in particular, and work towards an International Pediatric Liver Tumors Consensus Classification that would be required for international collaborative projects. Twenty-two pathologists and experts in pediatric liver tumors, including those serving as central reviewers for the COG, European Société Internationale d'Oncologie Pédiatrique, Gesellschaft für Pädiatrische Onkologie und Hämatologie, and Japanese Study Group for Pediatric Liver Tumors protocols, as well as pediatric oncologists and surgeons specialized in this field, reviewed more than 50 pediatric liver tumor cases and discussed classic and newly reported entities, as well as criteria for their classification. This symposium represented the first collaborative step to develop a classification that may lead to a common treatment-stratification system incorporating tumor histopathology. A standardized, clinically meaningful classification will also be necessary to allow the integration of new biological parameters and to move towards clinical algorithms based on patient characteristics and tumor genetics, which should improve future patient management and outcome.

Modern Pathology (2014) 27, 472–491; doi:10.1038/modpathol.2013.80; published online 6 September 2013

Keywords: hepatoblastoma; pathology; pediatric liver cancer classification; prognosis

Correspondence: Dr D López-Terrada, MD, PhD, Department of Pathology, Texas Children's Hospital, Baylor College of Medicine, 6621 Fannin St, Suite AB1195, Houston, TX 77030, USA.

E-mail: doloresl@bcm.edu

Received 28 January 2013; revised 28 February 2013; accepted 5 March 2013; published online 6 September 2013

Liver cancer is rare in children, with only 1.5 cases per million children under 18 years of age throughout the world. The majority of pediatric liver tumors are sporadic, but they can also be associated with familial cancer syndromes, metabolic disorders and predisposing conditions, such as extreme prematurity and low birth weight.^{1,2} The rarity of these tumors, even in specialized institutions, makes their diagnoses challenging for the general pathologist, particularly because of the lack of a current international consensus classification. Furthermore, it is difficult, even for a national group of investigators, to assemble enough patients to undertake controlled therapeutic trials and large biological studies.

With the introduction of the pediatric collaborative therapeutic protocols and systematic central histopathological review of pediatric liver tumors, the final diagnosis often relies on experienced, specialized pathologists, sometimes serving as central reviewers for cooperative groups such as Children's Oncology Group (COG), SIOPEL (Société Internationale d'Oncologie Pédiatrique, International Childhood Liver Tumors Strategy Group), GPOH (Gesellschaft für Pädiatrische Onkologie und Hämatologie), or JPLT (Japanese Study Group for Pediatric Liver Tumors). The COG pathology centralized review allowed the identification of histologic subtypes with distinct clinical associations and, as a result, histopathology was incorporated within its protocols as a risk-stratification parameter³⁻⁵ and as a critical component for patient management. However, this approach has been the exception until recently. Therefore, the COG Liver Tumor Committee sponsored an International Pathology Symposium in March 2011 to discuss the histopathology and classification of pediatric liver tumors, to share experience, and to work towards an International Pediatric Liver Tumors Consensus Classification that would be required for international collaborative projects. This work is also part of the Children's International Hepatic Tumors Collaboration initiative, also supported by the European Network for Cancer Research in Children and Adolescents European grant.

Twenty-two pathologists and experts in pediatric liver tumors, including those serving as central reviewers for all four cooperative groups, as well as pediatric oncologists and surgeons specialized in this field, reviewed 50 pediatric liver tumor cases and discussed classic and newly reported entities, and criteria for their histologic classification. This symposium represented the first collaborative step including international pathologists, oncologists and surgeons to develop a classification that may lead to a common treatment-stratification system incorporating tumor histopathology. A standardized, clinically meaningful classification will also be necessary to allow the integration of new biological parameters and to move towards clinical algorithms based on patient characteristics

and tumor genetics, which should improve future patient management and outcome.

This manuscript describes the most relevant pediatric liver tumor types and subtypes discussed during the symposium, in particular hepatoblastoma, their classification criteria, immunohistochemical panels, and other ancillary tools, as well as the recommendations for submission, sampling, and evaluation of diagnostic specimens, as proposed by this international collaborative group of pediatric liver tumor experts.

Pediatric liver tumor case submission and review

Fifty pediatric tumor cases were submitted by the participating pathologists from nine different countries. Hematoxylin & eosin-stained slides and immunohistochemical stains from selected cases were centrally scanned by the Biopathology Center (Nationwide Children's, Columbus, Ohio) using the Aperio system, and were made available for electronic review prior to the meeting. Cases selected included liver tumors from patients between 2 months and 18 years of age, and the following diagnoses: 24 hepatoblastomas, 14 epithelial type, including 8 fetal, 3 embryonal and fetal, and 3 cholangioblastic, 5 mixed epithelial and mesenchymal (including 3 teratoid cases), and 5 small-cell undifferentiated or with small-cell undifferentiated component. There were also hepatocellular tumors, other than hepatoblastoma submitted, including three hepatocellular carcinomas (one fibrolamellar, two well differentiated), one regenerative nodule, two cholangiocarcinomas, one mixed hepatocellular carcinoma–cholangiocarcinoma, two liver cell adenomas, and one case of adenomatosis. Ten other non-hepatocellular pediatric liver tumors submitted and reviewed by the group included two rhabdoid tumors, two epithelioid hemangioendotheliomas, one combined mesenchymal hamartoma/embryonal sarcoma, one pure embryonal sarcoma, one inflammatory myofibroblastic tumor, one angiosarcoma, and one nested stromal epithelial tumor of the liver.

An independent case review demonstrated a high degree of consensus among the participating pathologists regarding common histologic hepatoblastoma subtypes (epithelial, mesenchymal, fetal, and embryonal), as well as regarding cholangioblastic and teratoid variants. However, the presence of a minor small-cell-undifferentiated component (three cases) was only recognized by 8 of 15 participants, whereas consensus was high (12 of 15) for cases with >50% small-cell component. Other diagnoses lacking consensus included two cases submitted as anaplastic fetal hepatoblastoma (85% of the participants either did not submit a response or classified them as hepatocellular carcinoma) and one case submitted as combined hepatoblastoma/hepatocellular carcinoma (seven of the participants diagnosed it as

carcinoma, five as hepatoblastoma, and four as transitional cell liver tumor). Only 8 of the 15 reviewers agreed on the diagnosis of regenerative nodule *versus* well-differentiated hepatocellular carcinoma. Diagnostic consensus for these and other non-hepatocellular neoplasms (particularly sarcomas) was challenged by the lack of immunohistochemical stains, lack of or insufficient clinical history (as many were cases seen in consultation or centrally reviewed), and sampling limitations (small, limited biopsies), often proven inadequate to accurately classify these tumors.

Common pediatric liver malignancies: hepatoblastoma and its histological variants

Approximately two-thirds of all liver masses occurring in children are malignant, and almost 70% of those are hepatocellular in origin, either hepatoblastomas or hepatocellular carcinomas.^{2,6} Hepatoblastoma, usually diagnosed during the first 3 years of life, and the most common pediatric liver malignancy, is an embryonal tumor believed to arise from a hepatocyte precursor that often recapitulates stages of liver development. These tumors are rarely composed of only one cell type, usually demonstrating combinations of epithelial, mesenchymal, undifferentiated, and other histologic components^{6,7} (Tables 1 and 2). The most common one is the embryonal pattern that resembles the liver at 6–8 weeks of gestation. Embryonal hepatoblastoma cells (Figure 1c) are ~10–15 μ in diameter, round or more commonly angulated, with scant cytoplasm, high nuclear–cytoplasmic ratio slightly higher than that of non-neoplastic hepatocytes, and grow in sheets or are organized forming tubular or acinar formations around a central lumen.

In addition to other epithelial components described below, 20–30% of hepatoblastoma specimens also contain stromal derivatives, including spindle cells ('blastema'), osteoid, skeletal muscle, and cartilage, leading to the designation of 'mixed' hepatoblastomas. When there is a mixture of heterologous components (including endoderm, neuroectodermal derivatives, melanin-containing cells, and other elements), tumors are classified as 'teratoid' hepatoblastomas.⁸ The prognostic significance of this variant is at the moment still uncertain.

Well-differentiated fetal hepatoblastoma (or pure fetal hepatoblastoma with low mitotic activity)

Well-differentiated fetal hepatoblastoma is composed of cells measuring between 10 and 20 μ in diameter that grow either as one- to two-cell thick cords forming slightly more cellular trabeculae or in

Table 1 Pediatric liver tumors consensus classification

<i>Epithelial tumors</i>
Hepatocellular ^a
Benign and tumor-like conditions
Hepatocellular adenoma (adenomatosis)
Focal nodular hyperplasia
Macroregenerative Nodule
Premalignant lesions
Dysplastic nodules
Malignant
Hepatoblastoma
Epithelial variants
Pure fetal with low mitotic activity
Fetal, mitotically active
Pleomorphic, poorly differentiated
Embryonal
Small-cell undifferentiated
INI1-negative
INI1-positive
Epithelial mixed (any/all above)
Cholangioblastic
Epithelial macrotrabecular pattern
Mixed epithelial and mesenchymal
Without teratoid features
With teratoid features
Hepatocellular carcinoma
Classic HCC
Fibrolamellar HCC
Hepatocellular neoplasm NOS ^b
Biliary
Benign
Bile duct adenoma/hamartoma, other
Malignant
Cholangiocarcinoma
Combined (hepatocellular cholangiocarcinoma)
<i>Mesenchymal tumors</i>
Benign
Vascular tumors
Infantile hemangioma
Mesenchymal hamartoma
Pecomas
Malignant
Embryonal sarcoma
Rhabdomyosarcoma
Vascular tumors
Epithelioid hemangioendothelioma
Angiosarcoma
<i>Other malignancies</i>
Tumors of uncertain origin
Malignant rhabdoid tumor
INI1 – (documented <i>INI1</i> mut)
INI1 +
Nested epithelial stromal tumor
Other
Germ cell tumors
Teratoma
Yolk sac tumor
DSRCT
pPNET
Metastatic (and secondary)
Solid tumors (NB, Wilms, other)
Acute myeloid leukemia (M7)

Abbreviations: DSRCT: desmoplastic small round cell tumor, pPNET: peripheral primitive neuroectodermal tumor, NB: neuroblastoma.

Note: associated genetic syndromes, malformations, familial cancer syndromes, metabolic disorders, prematurity, and underlying liver disease should always be reported when available.

^aClassification applies only to pre-chemotherapy specimens.

^bIndicates provisional entity. Tumors previously designated as transitional liver cell tumors may be included in this category.

Table 2 Hepatoblastoma classification

<i>Epithelial</i>	
Fetal	'well-differentiated': uniform (10–20 μ in diameter), round nuclei, cords with minimal mitotic activity (<2 per $\times 10/400$ microscopic fields), EMH ^a 'crowded' or mitotically active (>2 per $\times 10/400$ microscopic fields); conspicuous nucleoli, less glycogen 'pleomorphic, or poorly differentiated' moderate anisonucleosis, high N/C, nucleoli 'anaplastic' marked nuclear enlargement and pleomorphism, hyperchromasia, abnormal mitoses
Embryonal	10–15 μ in diameter, high N/C, angulated nuclei, primitive tubules, EMH
Macrotrabecular	epithelial HB (fetal or embryonal) growing in trabeculae of >5 cells thick (between sinusoids)
Small-cell	5–10 μ in diameter, no architectural pattern, minimal pale amphophilic cytoplasm, round to oval nuclei with fine chromatin and inconspicuous nucleoli, +/– mitoses; +/– INI1 ^b
undifferentiated	
Cholangioblastic	bile ducts, usually at periphery of epithelial islands, can predominate
<i>Mixed</i>	
Stromal derivatives	spindle cells ('blastema'), osteoid, skeletal muscle, cartilage
Teratoid	mixed, plus primitive endoderm; neural derivatives, melanin, squamous and glandular elements

^aEMH, extramedullary hematopoiesis.

^bPure small-cell undifferentiated needs to be differentiated from malignant rhabdoid tumors (discohesive, eccentric irregular nuclei, prominent nucleoli, abundant cytoplasmic filaments including cytokeratin and vimentin, negative nuclear INI).

sheets.⁶ These cells have centrally placed, round, small nuclei, finely stippled chromatin, well-delineated nuclear membranes, and inconspicuous nucleoli. They resemble fetal hepatocytes and may contain variable amounts of glycogen or lipid, causing many of them to have clear cytoplasm (Figure 1a). They commonly contain clusters of hematopoietic precursors (extramedullary hematopoiesis).^{4,9} This pattern more commonly presents as a component of epithelial, or mixed epithelial and mesenchymal types, as described below.

Several studies have documented a correlation between well-differentiated fetal histology and better outcome,^{4,10,11} particularly for pure fetal hepatoblastoma with minimal mitotic activity (<2 per 10 high-power $\times 400$ microscopic fields).⁶ The importance of recognizing pure, well-differentiated fetal hepatoblastoma is that this is a surgically curable tumor, and chemotherapy is unnecessary when completely resected at diagnosis. All stage-I well-differentiated fetal hepatoblastoma patients with tumors showing low mitotic activity enrolled in the two most recent COG protocols have been cured by surgery alone.¹² Unfortunately, the diagnosis of well-differentiated fetal histology is not possible with small biopsies, or with post-chemotherapy specimens, as it requires evaluation of the complete resection specimen prior to chemotherapy.

Most international protocols (other than those of the COG) have historically recommended treating all children having liver tumors and elevated alpha-fetoprotein with chemotherapy before tumor resection, with histologic evaluation being limited to the biopsy specimen, whenever available, or post-chemotherapy specimen. Given the characteristic microscopic heterogeneity of hepatoblastoma, a biopsy that samples approximately <3/100 000 of the entire tumor (an estimated 15 mg *versus* 500 grams before treatment, on average) is only rarely (8–10%) representative of the rest of the

tumor.¹³ The post-chemotherapy surgical specimen usually demonstrates regressive and necrotic changes, and changes in the nuclei, sometimes making it difficult to evaluate the characteristics of persistent viable tumor. These changes may include ossification, squamous nests, or increased connective tissue, mimicking a mixed hepatoblastoma, or extensive cholangioblastic differentiation, less often found in pretreatment biopsies.¹⁴

Other types of fetal hepatoblastoma: crowded fetal (also known as mitotically active fetal)

A fetal cytologic pattern in hepatoblastoma may be well differentiated but mitotically active. According to the COG protocols, this pattern needs to be recognized and differentiated from the well-differentiated fetal pattern, as it necessitates chemotherapy. The term 'crowded fetal' is synonymous of 'mitotically active fetal' (more than two mitoses per 10 high-power fields, $\times 400$ microscopic fields). In this pattern, cells still show well-delineated plasma membranes but more amphophilic cytoplasm, and proportionately higher nuclear/cytoplasmic ratio, producing a 'crowded' appearance (Figure 1b). The nucleoli tend to be more prominent, and increased mitoses are present. Rarely this may be the dominant pattern but is usually intermixed with well-differentiated fetal areas when its recognition is essential to prompt the use of chemotherapy, according to the current COG protocols. Glypican 3 immunohistochemistry can be useful to differentiate well-differentiated fetal from other fetal patterns because of the relatively finely granular positivity in the less mitotically active cells. Crowded fetal is often adjacent to embryonal areas (and small-cell undifferentiated), and a transition between the two

Figure 1 (a) Well-differentiated fetal hepatoblastoma; (b) crowded (mitotically active) fetal pattern; (c) embryonal hepatoblastoma; (d) pleomorphic hepatoblastoma; (e) cholangioblastic differentiation; and (f) macrotrabecular pattern.

patterns can be found frequently. Although the documentation of the relative proportions of different hepatoblastoma components, including crowded fetal pattern, has been suggested, it is not clear at the

moment whether the presence, or what proportion of any epithelial pattern other than pure well-differentiated fetal and small-cell undifferentiated, will be associated with prognosis.

Table 3 Immunohistochemical stains useful for the diagnosis of hepatoblastoma

	<i>Fetal</i>						
	<i>WDF</i>	<i>Fetal with mitoses</i>	<i>Pleomorphic</i>	<i>Embryonal</i>	<i>Small-cell undifferentiated</i>	<i>Mesenchymal</i>	<i>Cholangioblastic</i>
GPC3	Finely granular	+++ Coarse	++ Coarse	+++ Coarse/rare	–	–	–
Beta-cat	Variably +/+ + + nuclear or normal	+/+++ Nuclear	+/+++ Nuclear	+/+++ Nuclear, can be –	+++ Nuclear	+++ Nuclear on osteoid/blastema/neg in teratoid elements	Variable/positive nuclei
GS	+++	+++	Variable	Variable, can be neg	–	–	–
Hep Par	+++	+++	Variable	Usually –	–	–	–
Cyclin D1	–	+/+++	+/+++	+/+++	+/+	Variable/neg in teratoid	–
CK7	–	–	–	–	–/+	Variable/weak in blastema	+++
CK19	–	–	–	–	+/+++ Variable	Neg/weak in blastema	+++
CD34	+ Endo	+ Endo	–	–	+/+++	+++	Usually –
Vimentin	–	–	–	–	+/+++	+++	+++
INI1	+++	+++	+++	+++	Negative in pure SCU; variable when mixed	+++	+++

Pleomorphic component in epithelial hepatoblastoma

A pleomorphic epithelial pattern is an uncommon pattern of hepatoblastoma, more often seen in post-chemotherapy specimens and in metastases following chemotherapy. Individual cells in pleomorphic hepatoblastoma pattern retain their fetal or embryonal appearance because of their polygonal shape and often abundant eosinophilic cytoplasm, but show nuclear features that are more pleomorphic as compared with well-differentiated fetal or crowded fetal patterns, with variably coarse chromatin, irregular shape, and large, conspicuous nucleoli (Figure 1d). Although in most examples these cells more closely resemble fetal hepatoblasts, hepatoblastomas with embryonal pattern may also show pleomorphism, and hence the term ‘hepatoblastoma with pleomorphic epithelial pattern’ is preferred. Even though mitoses are frequently found in pleomorphic epithelial hepatoblastomas, these do not display classic features of ‘anaplasia’, such as large cell size (three to four times that of adjoining cells) and atypical multipolar mitoses, which occur very rarely in hepatoblastoma and to date has uncertain prognostic relevance.

When these pleomorphic cells assume a macrotrabecular pattern of growth (see below) (Figure 1f), tumors may be difficult to distinguish from hepatocellular carcinoma. A good example is patient 5 in the report by Prokurat *et al*,¹⁵ whose post-chemotherapy specimen resembles clear cell hepatocellular carcinoma; however, as the patient responded completely to chemotherapy, the true nature of the tumor is uncertain. In the terminology proposed by Prokurat *et al*,¹⁵ this was termed a ‘transitional liver cell tumor’, although the consensus at the Los Angeles symposium was in favor of ‘pleomorphic epithelial hepatoblastoma’.

The presence of more typical hepatoblastoma areas elsewhere in the tumor can be helpful in such instances; however, both forms of hepatocellular neoplasia can occur together.²

The pleomorphic epithelial pattern has been also referred to in the past as ‘anaplastic fetal’ and ‘hepatocellular carcinoma-like’, which are confusing terms as they may suggest a different tumor biology. Hence, the term ‘pleomorphic epithelial’ was preferred by the consensus group, and the term ‘anaplasia’ was reserved for features such as those used for Wilms tumor, as described above. Future studies will be necessary to evaluate the significance of the presence of this pattern.

Cholangioblastic hepatoblastoma

In a subset of hepatoblastomas, some of the neoplastic cells differentiate as cholangiocytes and form small ducts.^{16,17} This cholangiocellular component expresses cholangiocyte lineage markers (cytokeratins 7 and 19) and may be situated within or surrounding the hepatocellular component of the tumor. This component needs to be differentiated from tubular or acinar structures found in embryonal hepatoblastoma, which are typically small with less cytoplasm, more mitotically active, and express glypican 3, whereas the cholangiocellular component is usually negative (see Table 3). The cells lining these ductular components tend to be cuboidal rather than columnar, as those seen in ducts, and the nuclei are usually round with coarse chromatin (Figure 1e). Beta-catenin staining can be very useful, especially in post-chemotherapy specimens when reactive ductal proliferation is common, as neoplastic ducts usually demonstrate nuclear staining, as opposed to membranous expression of benign ducts (Figure 4e).

Figure 2 (a, b) Hepatoblastoma with nests of small-cell component; (c) INI1 immunohistochemistry demonstrating nuclear positivity of the SC component; (d) primary rhabdoid tumor of the liver in an infant; (e) positive vimentin; and (f) negative INI1 immunostaining.

The differential diagnosis of cholangioblastic hepatoblastoma includes other cholangioblastic tumors of childhood, such as the very rare so-called ductal plate tumors and pediatric intrahepatic cholangiocarcinoma, both of which are easily distinguishable from cholangioblastic hepatoblastoma, as the first usually closely recapitulates ductal plate structures and the second is an exquisitely desmoplastic tumor, unlike cholangioblastic hepatoblastoma.¹⁸ Another relatively recently described tumor entity that may need to be considered in the differential diagnosis is the nested stromal epithelial tumor of the liver,¹⁹ also termed desmoplastic nested spindle-cell tumor of the liver.²⁰ This is a rare mixed tumor variant with a distinctive pattern of epithelial nests in a spindle-cell stroma, often showing calcification or ossification in close association with bland-appearing bile ducts.¹⁹ So far, the relationship of nested stromal epithelial tumor to other ‘blastomal’ hepatic tumors of infancy and childhood remains unclear.

Small-cell-undifferentiated hepatoblastoma, hepatoblastoma with small-cell component

Hepatoblastomas may contain undifferentiated small cells, sometimes coexpressing cytokeratin and vimentin, reflecting neither epithelial nor stromal differentiation. This component was originally reported as ‘anaplastic type’ but had been replaced by the term ‘small-cell undifferentiated’.^{4,6,21} Small cells are histologically slightly larger than

lymphocytes (usually 7–8 μ), round to oval with scant cytoplasm, with relatively fine nuclear chromatin, inconspicuous nucleoli, and only minimal mitotic activity (Figure 2a). They may grow in a diffuse pattern but usually form clusters either intimately intermixed with epithelial cell types or forming nests in an almost ‘organoid’ pattern (Figure 2b). Identification of this pattern may be missed because of inadequate sampling or may be interpreted as embryonal or as blastemal cells, especially when the nuclei are ovoid. Some specimens may show a loose myxoid background and have a microcystic pattern of arrangement overlapping with embryonal areas. Immunohistochemically, small cells show variable immunoreactivity for pancytokeratin, cytokeratins 8 and 18, and vimentin, and do not express alpha-feto protein or glypican.²² Rarely, particularly in infants, the entire hepatoblastoma is composed of this small-cell type (small-cell-undifferentiated hepatoblastoma), which accounts for <5% of all hepatoblastoma.

Small-cell-undifferentiated hepatoblastoma belongs to the clinically important group of hepatoblastomas that show low or normal serum AFP levels,^{23,24} and it has been shown to be associated with an aggressive biology^{3,25,26} and worse survival.^{4,5,24} A recent report from the COG has shown that small-cell-undifferentiated histology is a prognostic factor for an increased risk of death.^{27–29}

Recently, it has become apparent that some small-cell-undifferentiated hepatoblastomas may present morphological and biological features characteristic of malignant rhabdoid tumors, such as the lack of INI1 nuclear expression,^{24,30–32} and the transition

between the two types has been described.⁶ In a recent clinical study of hepatoblastoma patients with small-cell-undifferentiated histology and normal or slightly elevated serum alpha-feto protein, some revealed no detectable INI1 nuclear staining.²⁴ It is important to recognize this variant, as these patients may benefit from a chemotherapy strategy designed for malignant rhabdoid tumors rather than hepatoblastoma. Malignant rhabdoid tumors with characteristic histomorphology can also occur as primary tumors in the liver, with large round to oval cells with vesicular nuclei, prominent nucleoli, and paranuclear inclusions containing intermediate filaments (rhabdoid cells) (Figures 2d–f).^{18,33}

In terms of prognosis, it will be important to differentiate INI1-negative and -positive small-cell components when admixed with other epithelial types, as the INI1-expressing form may not imply a worse prognosis. Symposium participants agreed upon a recommended panel of immunohistochemical stains, including pancytokeratin, vimentin, and glypican3, to help characterize and even detect small-cell-undifferentiated areas, as it may not be obvious in some cases. For tumors lacking INI1 nuclear expression, the diagnosis of rhabdoid-like tumor should be strongly favored, and morphology should be reviewed carefully for the presence of rhabdoid features (discohesive, eccentric irregular nuclei, prominent nucleoli, and abundant cytoplasmic filaments). INI1-negative tumors should also be submitted for mutation and deletion testing, and for patients screened for germ-line mutations (and family-counseled), whenever appropriate. However, it is important to remember that mutations in genes other than *INI1* have been reported in this group of tumors, and the standard of diagnosis for rhabdoid tumors is still histomorphology.³¹

An important issue to consider while evaluating small-cell-undifferentiated hepatoblastomas is the size and characteristics of the biopsy sample requirements to conduct this diagnosis (see 'sample submission' section). Recommendations made by the participants included estimating and documenting the percentage of small-cell-undifferentiated component upon review, and evaluation of the indicated immunohistochemical panel by pathologists at the local institutions, especially on resection specimens. According to the current COG protocol, the presence of any small-cell-undifferentiated component in a biopsy necessitates more extensive therapy. Documenting the percentage of this component will be important for the study, as collected data analysis may indicate whether an adverse prognosis is associated with any amount of small-cell undifferentiated or with a certain threshold amount. In addition, the submission of insufficient or suboptimal tissue specimens required for diagnosis also needs to be reflected in the reports and study protocol.

Macrotrabecular pattern

A macrotrabecular growth pattern, similar to that typically seen in hepatocellular carcinoma, may be present in a minority of hepatoblastomas, accounting for <5% of all cases. Macrotrabecular hepatoblastoma was first reported by Gonzalez-Crussi *et al*,²¹ who documented a growth pattern characterized by cell plates ≥ 20 -cell thick that could be found pure or in combination with other patterns. Cells within these macrotrabeculae may be fetal, embryonal, or pleomorphic and may be similar to those seen in hepatocellular carcinoma⁴ (Figures 1f and 3b). Zimmermann³⁴ proposed dividing macrotrabecular hepatoblastomas into two categories, those composed of hepatocellular carcinoma-like cells (MT-1) and a second group composed of fetal and/or embryonal cells (MT-2), with the first group assumed to be biologically closer to hepatocellular carcinoma, and possibly prognostically unfavorable. However, this distinction is yet to be adopted by others. Clinical data are currently insufficient to determine the prognostic significance of macrotrabecular pattern in hepatoblastoma.^{4,17,18,35}

Participants in the symposium recommended modifying the original macrotrabecular hepatoblastoma criteria, requiring the presence of ≥ 5 - to ≥ 20 -cell-thick -trabeculae. They also agreed on the importance of documenting the presence of focal macrotrabecular pattern and cell type, differentiating tumors with a predominant or exclusive macrotrabecular pattern from hepatocellular carcinomas and the need to perform molecular analyses and characterize the biology of this group of tumors to enable their distinction in future clinical trials.

Other hepatocellular tumors diagnosed in children

Hepatocellular carcinoma

Hepatocellular carcinoma represents ~20% of all malignant liver tumors diagnosed in children^{6,7} and constitutes a clinically challenging group often presenting as large, unresectable lesions, typically in an older children/adolescent population. Pediatric hepatocellular carcinoma includes a biologically diverse group of neoplasms, sometimes associated with underlying metabolic and/or genetic abnormalities¹³ that can occur in the first decade of life, and others similar to those diagnosed in adults. A second group of pediatric hepatocellular carcinomas arises in livers without chronic disease. These tumors may demonstrate a morphologic spectrum, sometimes overlapping with hepatoblastoma (Figure 3a). Immunohistochemical stains combined with histopathology are useful to differentiate hepatocellular carcinoma from other tumors, but not always from hepatoblastoma.¹⁸ Fibrolamellar hepatocellular carcinoma constitutes a distinct clinical and

Figure 3 (a) Well-differentiated hepatocellular carcinoma in a child with perinatally acquired hepatitis B virus infection; (b) hepatocellular carcinoma, macrotrabecular pattern; (c) hepatocellular carcinoma, fibrolamellar variant; and (d) Hepatocellular Neoplasm NOS.

histological variant representing almost one-third of all hepatocellular carcinomas diagnosed in patients under 20 years of age, characteristically without cirrhosis or underlying liver disease.^{36,37} This variant of hepatocellular carcinoma is characterized by large, eosinophilic (oncocytic) hepatocytes with prominent nucleoli embedded within lamellar fibrotic tissue (Figure 3c). Tumor cells express biliary, hepatocytic, and hepatic-progenitor markers and carry fewer genomic and epigenetic alterations than classic hepatocellular carcinoma.³⁷

Hepatoblastoma chemotherapy is unsuccessful in treating pediatric hepatocellular carcinoma, and the intensification of current agents does not result in any outcome improvement.^{38,39} Data from Société Internationale d'Oncologie Pédiatrique trials and other recent international therapeutic experience, suggest a higher chemotherapy response rate in pediatric than in adult hepatocellular carcinoma.^{38,40} For those cases that show chemoresistance, or to augment partial chemosensitivity, new therapeutic agents for adult hepatocellular

carcinoma have been proposed to treat pediatric hepatocellular carcinoma and refractory hepatoblastoma, such as Sorafenib and other kinase inhibitors.⁴¹ Given therapeutic and prognostic differences, it is crucial to differentiate pediatric hepatocellular carcinoma from hepatoblastoma and to investigate the underlying biology of this group of tumors, and whether pediatric hepatocellular carcinomas are biologically different or overlap with adult hepatocellular carcinoma (Table 4). There is no evidence at the moment supporting different therapeutic strategies to treat clinical and histological subtypes of pediatric hepatocellular carcinoma. The fibrolamellar variant does not appear to be clinically different (any better) from other subtypes of hepatocellular carcinoma⁴² but, if resectable, is associated with a better 5-year survival (~55%) probably because of the absence of underlying liver disease in these patients.⁴³

One of the challenges to study the biology of pediatric hepatocellular carcinoma, has been the limited numbers of cases and the lack of banked

Table 4 Most common cytogenetic and molecular abnormalities in pediatric liver neoplasms

Tumor type	Cytogenetic abnormalities	Molecular (mutations/pathways)
Hepatoblastoma (HB)	Trisomy 2, 8, 20, 22 gains Translocation t(1;4)(q12;q34) and rearrangements involving 1q, 2 and 4q	Wnt pathway (<i>CTNNB1</i> mutations (rarely <i>APC</i> , <i>AXIN</i>) Other pathways: SHH, Notch, PI3K/AKT and MAPK, IGF Expression (16 gene signature) and miRNAs
Hepatocarcinoma (adults ^a)	Gains: 1q, 8q, 6p, 9p, 10p Losses: 1p, 4p, 4q, 5q, 6q, 8p, 13q, 10q, 16p, 16q, 17p	Wnt, SHH, TGF β , MAP/ras, IGF, apoptosis, microsatellite instability, PTEN, p53, RB1.
Fibrolamellar carcinoma	Gains: 1q, 4q, 6p, 7p, 7q, 8q and 19p, and losses of 8p, 9p, 13q, 16p, 18q and Xq	DPC4/Smad4 loss, RAS, MAPK, PI3K, TGF- β , NF- κ B
Hepatocellular adenoma (adults ^a)	Gains: 1q and losses 6p +, 6q-, 11p, 13q, 17p	<i>HNF1A</i> -mutated (35–40%), steatosis, (biallelic-inactivating mutations of <i>HNF1A</i> gene, <i>FABP1</i> negative) <i>CTNNB1</i> -mutated (10–15%) (GS overexpression and nuclear beta-catenin) <i>Inflammatory HCA</i> (50%) gp130 binding site deletions (<i>IL6ST</i> gene) in ~60%. MALAT-1/MLHB1 fusion gene
Mesenchymal hamartoma	t(11;19)(q11;q13)	
Undifferentiated sarcoma (embryonal)	t(11;19)(q11;q13.4) (also in 1 mesenchymal hamartoma) Aneuploidy	
Hemangioma, infantile	del 6q(q21-q27)	1 case
Hemangioendothelioma		
Epithelioid hemangioendothelioma	t(1;3)(p36.3;q35)	WWTR1-CAMTA1 fusion
Malignant rhabdoid tumors	chromosome 22 abnormalities	<i>hSNF5/INI1</i> gene deletion or mutation

^aAbnormalities reported in adult hepatocellular adenomas and carcinomas; abnormalities in pediatric lesions have not been characterized.

specimens, as currently there are no therapeutic protocols or on-going pediatric clinical trials because of their rarity, highlighting the importance of collaborative and systematic efforts to collect and study these tumors, as well as integrating clinical, pathological, and biological data. A recent review of the international therapeutic experience with pediatric hepatocellular carcinoma supports earlier European findings that pediatric hepatocellular carcinoma has a higher rate of chemosensitivity than its adult counterpart and underscores the importance of future efforts to define the underlying biological differences between *de novo* pediatric tumors and adult tumors with cirrhosis that might account for this finding.^{38,40}

New Provisional Entity: Hepatocellular Malignant Neoplasm, NOS

For a small number of malignant hepatocellular tumors, consensus could not be reached by the reviewers. These tumors demonstrated several morphologies (Figure 3d) and, in some cases, a mixture of histological patterns typical of both hepatoblastoma and hepatocellular carcinoma (Figure 3a) in the same tumor, precluding their exact classification. Accurate diagnoses were also challenged by other factors, such as limited amount of specimen available for review, lack of immunohistochemical stains, other ancillary studies, or clinical information, such as age, underlying liver disease (metabolic disease, hepatitis, or cirrhosis), or preconditions. In order to capture this difficult-

to-classify group or tumors, the participants proposed the creation of a new 'hepatocellular malignant neoplasm NOS' provisional category that will need to be further characterized.

The term 'transitional cell liver tumors' was used by Prokurat *et al*¹⁵ to describe a series of seven highly malignant epithelial liver tumors diagnosed in older children and young adolescents, two of which were classified as hepatoblastomas based on biopsy findings, but with an unusual histopathology and poor response to chemotherapy. Clinically, these were large tumors at diagnosis, mostly on the right liver lobe, with high-serum alpha-fetoprotein levels and aggressive behavior. These tumors varied considerably histologically, with mixtures of cells growing in a solid/diffuse highly invasive pattern, and a complex mixture of hepatoblastoma-like cells resembling fetal, fetal-pleomorphic and/or embryonal cells, hepatocellular carcinoma-like cells, and poorly differentiated medium to large cells. Immunohistochemically, this group of tumors demonstrated variable cytoplasmic or membranous beta-catenin, and AFP positivity was often seen in hepatoblastoma, as well as in hepatocellular carcinoma-like cells, and cytokeratin 7 and/or cytokeratin 19, and EMA-positive cells were found at the periphery of the tumor nodules. Prokurat *et al*¹⁵ proposed the term transitional liver cell tumor based on the hypothesis that these tumors may represent a new type, with a putative cell-of-origin situated at a transition between hepatoblast and hepatocyte lineages. Whether these are clonally progressed hepatoblastomas, changes because of

Figure 4 (a) Glypican 3 well-differentiated fetal hepatoblastoma (20 ×); (b) glypican 3 embryonal component (40 ×); (c) Hep-Par1 fetal positive hepatoblastoma (40 ×); (d) β-catenin fetal and embryonal hepatoblastoma (40 ×); (e) β-catenin-positive cholangioblastic hepatoblastoma (40 ×); (f) CD34 in macrotrabecular hepatoblastoma.

chemotherapy, or rather a separate group of neoplasms of true transitional cell origin is unclear and will require further investigation. As a result, as the consensus at the Los Angeles symposium was to abandon the term transitional cell tumor of the liver in favor of the term 'hepatocellular malignant neoplasm'. The existence of this group of difficult-to-classify pediatric hepatocellular tumors highlighted the importance of complying with the specimen submission and clinical information requirements (see Specimen submission section) to facilitate correct diagnosis as well as enrollment in clinical trials, and to facilitate biological studies. It is important to remember that these classification criteria apply only to pre-chemotherapy specimens, as post-chemotherapy¹⁴-induced changes are under review (matched pre- and post-chemotherapy specimens) and will be the subject of future international collaborative efforts. The relevance of reporting other parameters that may be worth capturing, such as the proportion of histological components other than pure fetal and SC in pre- and post-chemotherapy specimens, or the percentage of necrosis,⁴⁴ is still unclear, and its true value will have to be determined by larger correlative studies.

A comprehensive histopathology review of pediatric hepatocellular carcinoma and other malignant pediatric hepatocellular liver tumors, including some previously classified transitional cell liver tumors, has been the subject of other recent international collaborative meetings in Paris and Gdansk during 2012, and the conclusions will be reported and discussed in a separate manuscript (Paris October 2011, and Gdansk May 2012 meetings).

Benign Hepatocellular Tumors (Adenomas, Adenomatosis)

Hepatocellular adenomas are rare benign tumors in children, most commonly diagnosed in adolescents in association with contraceptive use, but also with other conditions such as glycogen-storage disease, diabetes, androgen therapy, immunodeficiencies, and familial adenomatous polyposis.^{2,7} Lesions are usually solitary but may present as multiple adenomas ('adenomatosis').⁴⁵ Hepatic adenomas in children may demonstrate steatosis or fibrosis, and may be difficult to distinguish from other benign lesions such as focal nodular hyperplasia, typically a single large mass in a healthy liver and characterized by central scarring, or well-differentiated carcinoma, which may require characterization by immunohistochemistry and molecular analyses.⁴⁶ Recent molecular characterization of adult hepatocellular adenomas has demonstrated the existence of subgroups with strong genotype-phenotype correlations (Table 4),⁴⁷ which should also be applied to hepatic adenomas diagnosed in children. Interestingly, adenomas with beta-catenin mutations and Wnt activation, similar to those seen in hepatoblastomas, have a higher risk of malignant transformation and are associated with hepatocellular carcinoma in adults.⁴⁶

Other Pediatric Liver Tumors

Neoplasms other than malignant hepatocellular tumors (hepatoblastoma and hepatocellular carcinoma) are rare in children, with benign vascular

tumors being the most common, followed by sarcomas, mesenchymal hamartoma (10%), benign hepatocellular lesions (adenomas and focal nodular hyperplasia, 7%), and other tumors (4%).^{2,7,48} Malignant tumors of mesenchymal origin represent ~10–15% of all liver tumors diagnosed in children, according to the largest published series.⁶ This section will not attempt a comprehensive review of this rare group of tumors, but rather highlight some of the most distinct malignant entities, as well as some important differential diagnostic issues discussed during the symposium.

The most common form of vascular proliferations in children is infantile hemangiomas, usually spontaneously regressing.^{49,50} Infantile hepatic hemangiomas are classically seen on radiographic imaging as highly vascular rim-enhancing lesions and may present with elevated serum alpha-feto protein, and occasionally be mistaken for hepatoblastoma.⁵¹ Three clinical subgroups of infantile hepatic hemangioma—focal, multifocal, and diffuse—are recognized by the Liver Hemangioma Registry of the Vascular Anomalies Center at the Children's Hospital of Boston.⁵² Occasionally, the multifocal and diffuse clinical subtypes will show increased pleomorphism, intravascular spread, necrosis, and hemorrhage histologically, as well as faster growth. A few infantile hemangiomas have transformed into angiosarcomas in older children.⁵³ Epithelioid hemangioendothelioma is very rarely seen in children.⁵⁴

The most common sarcoma of the liver in children is embryonal sarcoma—a very aggressive neoplasm with a peak of incidence between 6 and 10 years. A dot-like cytoplasmic-positive staining for cytokeratin together with membranous CD56 reactivity and negative staining for myogenin are useful in the distinction of embryonal sarcoma from rhabdomyosarcoma and other hepatic sarcomas. Hepatobiliary rhabdomyosarcoma also occurs in children under 5 years of age and may demonstrate glypican 3 positivity.⁵⁵ Some embryonal sarcomas share a 19q13.4 chromosomal rearrangement and the translocation t(11;19) with mesenchymal hamartoma (Table 4)⁵⁶ that usually affect infants and children in the first 2 years of life and in the past was considered a developmental disorder. However, the identification of the same translocation t(11;19)(q13;q13.4) in some cases has demonstrated its neoplastic nature.

Another highly aggressive neoplasm primarily affecting infants and that needs to be mentioned is the malignant rhabdoid tumor. Classic histologic features of rhabdoid tumors include sheets of round to polygonal cells with large, vesicular nuclei, showing finely dispersed chromatin, a prominent eosinophilic nucleolus, and abundant eosinophilic cytoplasm containing paranuclear cytoplasmic inclusions, as has been discussed previously in the small-cell-undifferentiated hepatoblastoma section. Immunostains demonstrate a polyphenotypic profile, with diffuse staining for vimentin and focal

positivity for cytokeratin, EMA, S100, neuroectodermal markers (CD99, NSE, and synaptophysin) and myoid markers (smooth muscle actin and muscle-specific actin). Loss of nuclear INI1 protein expression is characteristic of and related to the biallelic inactivation of the tumor-suppressor gene *INI1* (*SMARCB1*) on chromosome 22q11.2, as previously discussed (Table 4).

The extensive use of INI1 immunostaining, fluorescence *in situ* hybridization, and mutation analyses in rhabdoid tumors developing outside the liver has contributed to the identification of morphologic variants of rhabdoid tumors; some are characterized by a prominent primitive undifferentiated cellular component, mimicking Ewing sarcoma/PNET or other round cell tumors.⁵⁷ Rhabdoid tumors may also represent a differential diagnostic problem in the liver, particularly for the small-cell-undifferentiated variant of hepatoblastoma (as previously discussed) and other small round cell tumors, and immunohistochemical and genetic characterizations of these lesions are required for their diagnosis.

Immunohistochemistry and other ancillary studies

Immunohistochemistry is now being increasingly used in the diagnosis and classification of hepatoblastoma subtypes. A limited panel using at least alpha-fetoprotein, glypican 3, beta-catenin, glutamine synthetase, vimentin, cytokeratin (pankeratin), Hep-Par1, and INI1 are presently the most useful in this setting (Table 3). These stains can be used in both biopsy and resection specimens and are especially useful in post-chemotherapy specimens for persistence of tumor. Alpha-fetoprotein is most likely to be positive in areas of epithelial hepatoblastoma and is not expressed in mesenchymal tissue or small-cell-undifferentiated areas. Well-differentiated fetal hepatoblastoma, macrotrabecular, and anaplastic areas may not express this antigen. The limitation of this stain is that secreted protein frequently results in high-background serum staining, making interpretation difficult. Normal liver in very young infants may also show some alpha-fetoprotein expression in the hepatocytes.

Hep-par1 highlights the fetal hepatoblastoma component, but it can be absent in the embryonal hepatoblastoma component (Figure 4c) and is always negative in the small-cell undifferentiated and mesenchymal areas. It stains the normal liver parenchyma and hence this stain is not useful in differentiating tumor cells in small biopsy specimens. Glypican 3 has been a very reliable stain that highlights the epithelial components and is negative in normal liver, benign tumors, small-cell undifferentiated and mesenchymal components of hepatoblastoma (Figures 4a and b). Although this stain

does not help in differentiating hepatoblastoma from hepatocellular carcinoma, the pattern of fine granular cytoplasmic staining appears to be unique to the well-differentiated fetal pattern of hepatoblastoma and helps in its recognition even in the biopsy specimens. Crowded fetal and embryonal hepatoblastoma show a coarse-diffuse cytoplasmic staining pattern, more like the one seen in hepatocellular carcinoma. Hepatocytes in non-neoplastic cholestatic diseases such as familial cholestasis and macroregenerative hyperplasia may also express glypican 3.

Beta-catenin, a marker of the canonical Wnt pathway activation, has been extensively studied and implicated in the tumorigenesis of hepatoblastoma. *CTNNB1* mutations are found in over 80% of hepatoblastoma cases, and beta-catenin immunohistochemistry is extremely helpful because of the presence of nuclear-staining pattern in the neoplastic tissue (Figure 4d). Diffuse cytoplasmic expression without nuclear staining usually also implies neoplasia, as normal hepatocytes and biliary epithelial cells have distinct membranous staining only. A subset of adenomas and pediatric hepatocellular carcinomas also shows nuclear beta-catenin staining. Positive beta-catenin staining is present in both epithelial and mesenchymal hepatoblastoma components and is variable in small-cell undifferentiated. The staining may also be variable in well-differentiated fetal areas; however, some examples of well-differentiated fetal hepatoblastoma with extensive nuclear staining have been documented. The neoplastic cells of immature mesenchyme ('blastema') and osteoid also show strong nuclear staining, as do clusters of small-cell-undifferentiated areas within the epithelial hepatoblastoma. Teratoid areas do not show significant nuclear expression especially in the neuroepithelial components. Nuclear beta-catenin staining is also noted within the neoplastic cholangioles of a cholangio-blastic hepatoblastoma (Figure 4e); however, the staining pattern is only membranous and cytoplasmic within reactive bile ducts seen at the periphery of hepatoblastoma tissue.

Glutamine synthetase is normally expressed in a single layer of hepatocytes around the terminal hepatic (central) vein. In neoplasia, it is a marker for activated Wnt and also a differentiation marker in hepatoblastoma. It is therefore seen with high intensity in fetal hepatoblastoma but less frequently in embryonal, small-cell-undifferentiated and mesenchymal areas.

The expression of cytokeratins in hepatoblastoma is variable and highlights the epithelial component to varying degrees using a pancytokeratin. CK7 and CK19 are biliary markers and are usually negative in epithelial and mesenchymal hepatoblastoma components. Small-cell-undifferentiated areas will show some positive keratin inclusions. Vimentin usually highlights mesenchymal as well as the 'blastema' components. Small-cell-undifferentiated areas may

also show vimentin expression (Figure 2e); however, it is negative in the epithelial component. INI1, as previously discussed, is an especially useful stain to distinguish a population of usually pure small-cell-undifferentiated hepatoblastoma, as those showing loss of nuclear INI1 expression need to be recognized as behaving like malignant rhabdoid tumors (Figure 2f). Small foci of small-cell-undifferentiated area in a more typical hepatoblastoma, however, are usually INI1-positive. INI1 staining is retained in all other components of hepatoblastoma, although less intense in WD-fetal nuclei.

Karyotyping and genetic profiling have demonstrated the presence of a number of recurrent chromosomal abnormalities in hepatoblastoma, the most common being trisomies (chromosomes 2, 8, and 20), rearrangements involving 1q, 4q, 2, and 22,^{58–60} and double-minutes (Table 4). It has been suggested that chromosome 8 and 20 gains may be associated with an adverse prognosis; however, so far this has not been used to modify therapy.

Molecular mechanisms leading to hepatoblastoma include the activation of the developmental and oncogenic signaling pathways. The canonical Wnt pathway is often activated by acquired mutations of the beta-catenin (*CTNNB1*) gene and less commonly through constitutional mutations of the *APC* gene or somatic mutations of other genes in the pathway.^{61–63} Other pathways implicated in hepatoblastoma include Sonic Hedgehog, Notch, Hepatocyte Growth Factor/c-Met (PI3K/AKT and MAPK signaling activation), the Insulin-Like Growth Factor (IGF) pathway, and others^{64–69} (Table 4). Molecular profiling of hepatoblastoma^{70–74} has identified groups of tumors and gene signatures that appear useful to further stratify these patients.^{71,75}

Further characterization of these biological mechanisms and the integration of molecular and clinical parameters into current morphologic classifications will be necessary to accurately diagnose and stratify pediatric liver tumor patients, similarly to other pediatric malignancies. Clinical validation of these new molecular stratification tools using larger clinical data sets will also require international collaboration.

Common cytogenetic and molecular abnormalities reported in other pediatric liver tumors, and including aberrantly activated signaling pathways, are listed in Table 4.

Recommendations for submission, sampling, and evaluation of pediatric liver tumor diagnostic specimens

A diagnostic tumor biopsy should be mandatory for all patients regardless of their age and serum alpha-feto protein level,^{29,39,76,77} which is different from what has been historically practiced in some clinical trials. The aim of the biopsy is twofold: first, to

obtain adequate tumor material for histological diagnosis and classification, and second to obtain fresh and frozen tumor with matched adjacent normal liver for biological studies. The biopsy should be performed before chemotherapy is started, as the histologic and morphologic classification criteria presented here apply only to pre-chemotherapy specimens.

Regarding what tissue sample is required to make the diagnosis, the COG sample requirement guidelines have been previously published.⁷⁸ The current COG AHEP-0731 trial accepts percutaneous core needle biopsy, laparoscopic core needle or wedge biopsy, and/or open core needle or wedge biopsy. Choice of technique will depend upon patient characteristics, the size and location of the tumor, and the team available at the institution. Biopsy samples should be evaluated fresh by the pathologist, whenever possible. As biopsies of pediatric liver tumors present significant potential for diagnostic error, even on permanent sections, intraoperative frozen sections should be avoided. Fine-needle aspiration should also be avoided for diagnosis, as the material obtained is usually insufficient to evaluate tumors in this age group.

Vitally important is the submission of sufficient tissue to conduct the diagnosis. When biopsy is carried out using the core needle (preferably co-axial) technique, we recommend a bare minimum of 5, and preferably 10 cores measuring no less than 1×0.3 cm each and, where possible, representative of different areas of the tumor. A designated biopsy of the adjacent non-tumoral liver should also be submitted whenever possible. To prevent tumor seeding on the needle tract, the technique should be co-axial and the needle tract should traverse an area of liver that will be resected at the time of definitive tumor resection. This mandates careful pre-biopsy communication between the surgeon and the radiologist when biopsies are to be obtained by an interventional radiologist. Multiple needle biopsies may succeed in sampling of different areas of the tumor identified by imaging studies. Fresh viable cells are necessary for cytogenetic studies and frozen tumor material (ideally also of the host liver) for other biological studies. This is an important consideration, as the inherent complexity of these tumors, some of which are extremely rare, combined with the paucity of available material, has hindered research and limited the clinical stratification tools and treatment options for these patients. In large heterogeneous tumors, harvesting material from different areas is necessary but this may require either open biopsy or surgical excision before chemotherapy.

Biopsy recommendations should be understood by the multidisciplinary team (that is, clinicians, oncologists, surgeons, hepatologists, and interventional radiologists), and their participation is of utmost importance to achieve the correct diagnosis for tailoring individual patient treatment and for

assignment of the patient to the correct arm of a protocol. Collection of liver tumor specimens at diagnosis sufficient for biologic analysis is critically important to advance our therapeutic strategies for liver tumors. It is hoped that biologic prognostic factors will help to define therapy reduction strategies to avoid toxicity and therapy intensification strategies to improve outcome in future studies. Towards this goal, it is strongly recommended that patients'/parents' consent for participation in biologic studies is obtained prospectively and not retrospectively^{29,39} and that pathologists should be informed preoperatively regarding the consent status so that specimens can be aliquoted appropriately and at least one (and preferably three) of the tissue cores frozen for ancillary studies.

If definitive resection is performed at diagnosis, the entire tumor will be available for prechemotherapy analysis. Often, the definitive resection will be performed after chemotherapy. In either case, representative specimens of grossly different tumor areas should be submitted for diagnosis and frozen for biological studies. To assure sampling and identification of areas of unfavorable histology (for example, small-cell undifferentiated), the total number of sections taken should be at least equal to or greater than the greatest dimension of the tumor measured in centimeters. The samples from inked margins of resection and gross vascular involvement (portal vein or hepatic vein–inferior vena cava) should be submitted, and the site and extent of macroscopic vascular invasion should be carefully documented. In our attempts to validate the preoperative PRETEXT and POST-TEXT grouping of the tumor, the pathologist should document which Couinaud segments of the liver are pathologically involved by the tumor⁷⁹ (Figures 5 and 6). In multifocal tumors, the size and segment location of all tumor nodules should be reported; this is especially important in helping to identify the location of any nodules found on pathologic evaluation that were not radiographically apparent on preoperative imaging. Additionally, microscopic intravascular growth, and whether it is within the tumor mass or outside of it, should be recorded. Frozen sections are often prepared to evaluate margins of resection. Tissue processing should also include freezing ~1 g (a minimum of 100 mg) of tumor from each grossly different appearing area of the tumor, corresponding to areas sampled for histology, as well adjacent non-tumoral liver. Viable sterile tumor should also be submitted for cytogenetic studies whenever possible.

It is important to highlight that most of the time pathologists are dealing exclusively with small biopsy specimens for the diagnosis of pediatric liver tumors, including hepatoblastoma. How much this represents the rest of the tumor, and how reliable a biopsy is for diagnosing a tumor with such a common intrinsic heterogeneity, is often unclear. The current hepatoblastoma COG protocol, AHEP-

Figure 5 PRETEXT is distinct from Couinaud 8-segment (I–VIII) anatomic division of the liver. PRETEXT defines 4 ‘Sections’. Boundaries of each section defined by the right and middle hepatic veins, and umbilical fissure.

Figure 6 PRETEXT (Pretreatment Extent of Disease) and POST-TEXT (Post-treatment Extent of Disease) extent of liver involvement system.

Table 5 Hepatoblastoma staging and risk stratification

<i>Traditional COG (Evan's) Staging system</i>	<i>Current COG risk stratification</i>	<i>Current SIOPEL risk stratification</i>
<i>Stage I:</i> complete gross resection at diagnosis with clear margins	<i>Very low risk:</i> pure fetal histology, Stage I or II (resected at diagnosis) ^a	
<i>Stage II:</i> complete gross resection at diagnosis with microscopic residual disease at the margins of resection	<i>Low risk:</i> any Histology resected at diagnosis (stage I/II); see resection guidelines below ^a	<i>Standard risk:</i> PRETEXT I, II, III
<i>Stage III:</i> biopsy only at diagnosis or gross total resection with nodal involvement or tumor spill or incomplete resection with gross intra-hepatic disease	<i>Intermediate risk:</i> stage III tumors (includes SCU histology)	
<i>Stage IV:</i> metastatic disease at diagnosis	<i>High risk:</i> stage IV tumors, AFP <100 at diagnosis	<i>High risk:</i> PRETEXT IV, metastasis at diagnosis, SCU histology, AFP <100 at diagnosis

^aCOG surgical guidelines recommend resection of tumors at diagnosis (stage I/II) based upon PRETEXT. PRETEXT I and PRETEXT II resected at diagnosis if there is an anticipated >1 cm surgical margin based upon preoperative imaging.

0731, recommends primary up-front tumor resection in PRETEXT 1 and 2 tumors without major vessel involvement; therefore, chemotherapy can be adjusted to the histologic tumor type, which cannot be fully evaluated without histologic review of the resection specimen. The COG experience has demonstrated the importance of recognizing pure, well-differentiated fetal hepatoblastoma in resected specimens to avoid unnecessary chemotherapy to treat these patients, who have surgically curable tumors when completely resected.¹² Another reason to promote primary resection is recognizing the presence of small-cell-undifferentiated component, which may behave aggressively, even though we still do not have an answer to the consequences of having a small proportion of small-cell-undifferentiated area.

After chemotherapy, the role of the pathologist is to describe accurately and compare the pre-chemotherapy specimen to the post-chemotherapy specimen; therefore, there is an understanding of the response to therapy (for example, percent tumor necrosis), the pattern of resistance of the surviving cells, and the correlation with molecular genetic characteristics of the surviving cells (for example, expression of drug resistance).⁸⁰ Collaborative efforts to systematically review pre- and post-chemotherapy specimens, document the associated histological changes, understand their significance (such as percentages of epithelial and mesenchymal components, including of course small cell or necrosis in pre- and post-chemotherapy specimens), and establish potential diagnostic criteria for post-chemotherapy specimens are already on under way and will be the subject of subsequent reports.

It is critically important that sufficient clinical data be provided to the pathologist and ideally these data should accompany the specimen. Age, alpha-fetoprotein levels, underlying liver disease, Couinaud segments and vascular involvement on imaging studies (with and without contrast), PRETEXT/POST-TEXT designation including the VPEMC

component of the PRETEXT system, and with resection specimens, any area of concern for possible positive margin are all issues of upmost importance. Similarly, correlation with epidemiologic studies requires maternal and family histories, birthweight prematurity, underlying liver tumor-associated syndromes, and genetic, developmental, viral and metabolic disorders.

Summary and future plans

The International Pathology Liver Symposium hosted by the COG in Los Angeles in March 2011, and subsequent conferences in Paris (Société Internationale d'Oncologie Pédiatrique, Pediatric Liver Malignancies Biology Conference, October 2011), and Gdansk (Pathology Workshop in conjunction with the Gdansk International Pediatric Liver Tumors Consensus Meeting, April 2012) represent initial collaborative efforts to create a clinically meaningful consensus classification for pediatric liver malignancies. The Los Angeles symposium aimed to establish the basis for a hepatoblastoma classification and highlighted the necessity to carefully review pediatric hepatocellular carcinoma and other non-hepatoblastoma epithelial liver malignancies in subsequent conferences. This classification will facilitate international collaboration on future clinical trials and may improve standardization of treatment stratification according to tumor histopathology, similar to what COG protocols have already adopted (Table 5). The lack of diagnostic consensus in a few circumstances highlights the need for establishing clear diagnostic guidelines and morphologic criteria based on a consensus nomenclature, as well as for subsequent case review and working sessions by this international group of experts. These efforts will facilitate correlations between tumor histopathology and outcome by using data such as those collected by the Children's International Hepatic Tumors Collabora-

Figure 7 Los Angeles Pediatric Liver Tumors Symposium participants.

tion project database, as well as the creation of a consensus patient stratification system.

This on-going international effort will facilitate the progressive integration of new parameters and will allow us to move towards a classification and treatment risk assessment scheme based upon tumor and patient biology, which will improve patient stratification in the near future.

Acknowledgements

In addition to the authors, we want to acknowledge the valuable contributions of other pediatric liver tumors experts who participated in the symposium, including Drs Anita Gupta, Bruce Morland, Dinesh Rakheja, Hiroshi Horie, Jens Stahlschmidt, Jon Rowland, Kevin Bove, Marie-Anne Brundler, Marta Garrido, Takeshi Inoue, and Yukichi Tanaka (Figure 7). This project could not have been possible without the leadership and support of the Children's Oncology Group Liver Tumors and Rare Tumors Committees, and Drs Rebecca Meyers, Marcio H Malogolowkin and Carlos Rodriguez-Galindo, in particular. We also want to acknowledge the support provided by the European FP7 European Network for Cancer Research in Children and Adolescents grant (Grant Agreement No. 261474), and by the Biopathology Center (Nationwide Children's Hospital, Columbus, Ohio), which generously scanned the cases submitted, and made them available to all pathologists for electronic review. Lastly, we would like to express our sincere gratitude to Dr Alexander Judkins and other members of the Department of Pathology at Children's Hospital Los Angeles, for generously hosting the symposium, Dr James Versalovic at the Texas Children's Hospital Department of Pathology for his support, as well as Christie Hollins, Karen Prince, Kay Patel, Faith Hollingsworth, Drs Mika Warren, and Angshumoy Roy for

their valuable assistance with the organization of the symposium and the preparation of this manuscript.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

- 1 Darbari A, Sabin KM, Shapiro CN, *et al*. Epidemiology of primary hepatic malignancies in U.S. children. *Hepatology* 2003;38:560–566.
- 2 Finegold MJ. Hepatic tumors in childhood, In: Russo P, Ruchelli E, Piccoli DA (eds) *Pathology of Pediatric Gastrointestinal and Liver Disease*. Springer Verlag: New York, NY, USA, 2004.
- 3 Haas JE, Feusner JH, Finegold MJ. Small cell undifferentiated histology in hepatoblastoma may be unfavorable. *Cancer* 2001;92:3130–3134.
- 4 Haas JE, Muczynski KA, Krailo M, *et al*. Histopathology and prognosis in childhood hepatoblastoma and hepatocarcinoma. *Cancer* 1989;64:1082–1095.
- 5 Ortega JA, Douglass EC, Feusner JH, *et al*. Randomized comparison of cisplatin/vincristine/fluorouracil and cisplatin/continuous infusion doxorubicin for treatment of pediatric hepatoblastoma: A report from the Children's Cancer Group and the Pediatric Oncology Group. *J Clin Oncol* 2000;18:2665–2675.
- 6 Weinberg AG, Finegold MJ. Primary hepatic tumors of childhood. *Hum Pathol* 1983;14:512–537.
- 7 Stocker JT. *Hepatic Tumors in Children*, 2nd Edn (Ballistrieri Wed), Lippincott, Williams and Wilkins: Philadelphia, 2001.
- 8 Manivel C, Wick MR, Abenzoza P, *et al*. Teratoid hepatoblastoma. The nosologic dilemma of solid embryonic neoplasms of childhood. *Cancer* 1986;57:2168–2174.
- 9 Ishak KG, Glunz PR. Hepatoblastoma and hepatocarcinoma in infancy and childhood. Report of 47 cases. *Cancer* 1967;20:396–422.

- 10 Kasai M, Watanabe I. Histologic classification of liver-cell carcinoma in infancy and childhood and its clinical evaluation. A study of 70 cases collected in Japan. *Cancer* 1970;25:551–563.
- 11 von Schweinitz D, Gluer S, Mildenerberger H. Liver tumors in neonates and very young infants: diagnostic pitfalls and therapeutic problems. *Eur J Pediatr Surg* 1995;5:72–76.
- 12 Malogolowkin MH, Katzenstein HM, Meyers RL, *et al*. Complete surgical resection is curative for children with hepatoblastoma with pure fetal histology: a report from the Children's Oncology Group. *J Clin Oncol* 2011;29:3301–3306.
- 13 Hadzic N, Finegold MJ. Liver neoplasia in children. *Clin Liver Dis* 2011a;15:443–462; vii–x.
- 14 Wang LL, Filippi RZ, Zurakowski D, *et al*. Effects of neoadjuvant chemotherapy on hepatoblastoma: a morphologic and immunohistochemical study. *Am J Surg Pathol* 2010;34:287–299.
- 15 Prokurat A, Kluge P, Kosciesza A, *et al*. Transitional liver cell tumors (TLCT) in older children and adolescents: a novel group of aggressive hepatic tumors expressing beta-catenin. *Med Pediatr Oncol* 2002;39:510–518.
- 16 Libbrecht L, Desmet V, Roskams T. Stages of normal and aberrant intrahepatic bile duct development in a mixed hepatoblastoma. *Histopathology* 2003;42: 618–620.
- 17 Zimmermann A. Hepatoblastoma with cholangioblastic features ('cholangioblastic hepatoblastoma') and other liver tumors with bimodal differentiation in young patients. *Med Pediatr Oncol* 2002;39:487–491.
- 18 Zimmermann A, Lopez-Terrada D. Pathology of pediatric liver tumors. In: Zimmermann A, Perilongo G, Malogolowkin MH, Von Schweinitz D eds) *Pediatric Liver Tumors*. Springer: Berlin; 2011, pp 83–112.
- 19 Heerema-McKenney A, Leuschner I, Smith N, *et al*. Nested stromal epithelial tumor of the liver: six cases of a distinctive pediatric neoplasm with frequent calcifications and association with cushing syndrome. *Am J Surg Pathol* 2005;29:10–20.
- 20 Hill DA, Swanson PE, Anderson K, *et al*. Desmoplastic nested spindle cell tumor of liver: report of four cases of a proposed new entity. *Am J Surg Pathol* 2005;29: 1–9.
- 21 Gonzalez-Crussi F, Upton MP, Maurer HS. Hepatoblastoma. Attempt at characterization of histologic subtypes. *Am J Surg Pathol* 1982;6:599–612.
- 22 Zynger DL, Gupta A, Luan C, *et al*. Expression of glypican 3 in hepatoblastoma: an immunohistochemical study of 65 cases. *Hum Pathol* 2008;39:224–230.
- 23 De Ioris M, Brugieres L, Zimmermann A, *et al*. Hepatoblastoma with a low serum alpha-fetoprotein level at diagnosis: the SIOPEL group experience. *Eur J Cancer* 2008;44:545–550.
- 24 Trobaugh-Lotrario AD, Tomlinson GE, Finegold MJ, *et al*. Small cell undifferentiated variant of hepatoblastoma: adverse clinical and molecular features similar to rhabdoid tumors. *Pediatr Blood Cancer* 2009;52:328–334.
- 25 Douglass EC, Reynolds M, Finegold M, *et al*. vincristine, and fluorouracil therapy for hepatoblastoma: a Pediatric Oncology Group study. *J Clin Oncol* 1993; 11:96–99.
- 26 Feusner JH, Krailo MD, Haas JE, *et al*. Treatment of pulmonary metastases of initial stage I hepatoblastoma in childhood. Report from the Childrens Cancer Group. *Cancer* 1993;71:859–864.
- 27 Fuchs J, Rydzynski J, Von Schweinitz D, *et al*. Pretreatment prognostic factors and treatment results in children with hepatoblastoma: a report from the German Cooperative Pediatric Liver Tumor Study HB 94. *Cancer* 2002;95:172–182.
- 28 Maibach R, Roebuck D, Brugieres L, *et al*. Prognostic stratification for children with hepatoblastoma: The SIOPEL experience. *Eur J Cancer* 2012;48:1543–1549.
- 29 Meyers RL, Rowland JR, Krailo M, *et al*. Predictive power of pretreatment prognostic factors in children with hepatoblastoma: a report from the Children's Oncology Group. *Pediatr Blood Cancer* 2009;53: 1016–1022.
- 30 Gadd S, Sredni ST, Huang CC, *et al*. Rhabdoid tumor: gene expression clues to pathogenesis and potential therapeutic targets. *Lab Invest* 90:724–738.
- 31 Russo P, Biegel JA. SMARCB1/INI1 alterations and hepatoblastoma: another extrarenal rhabdoid tumor revealed? *Pediatr Blood Cancer* 2009;52:312–313.
- 32 Wagner LM, Garrett JK, Ballard ET, *et al*. Malignant rhabdoid tumor mimicking hepatoblastoma: a case report and literature review. *Pediatr Dev Pathol* 2007; 10:409–415.
- 33 Trobaugh-Lotrario AD, Finegold MJ, Feusner JH. Rhabdoid tumors of the liver: rare, aggressive, and poorly responsive to standard cytotoxic chemotherapy. *Pediatr Blood Cancer* 2011;57:423–428.
- 34 Zimmermann A. The emerging family of hepatoblastoma tumours: from ontogenesis to oncogenesis. *Eur J Cancer* 2005;41:1503–1514.
- 35 Conran RM, Hitchcock CL, Wacławski MA, *et al*. Hepatoblastoma: the prognostic significance of histologic type. *Pediatr Pathol* 1992;12:167–183.
- 36 El-Serag HB, Mason AC. Risk factors for the rising rates of primary liver cancer in the United States. *Arch Int Med* 2000;160:3227–3230.
- 37 Ward SC, Waxman S. Fibrolamellar carcinoma: a review with focus on genetics and comparison to other malignant primary liver tumors. *Semin Liver Dis* 2011; 31:61–70.
- 38 Gupta AA, Gerstle JT, Ng V, *et al*. Critical review of controversial issues in the management of advanced pediatric liver tumors. *Pediatr Blood Cancer* 2011; 56:1013–1018.
- 39 Zsiros J, Maibach R, Shafford E, *et al*. Successful treatment of childhood high-risk hepatoblastoma with dose-intensive multiagent chemotherapy and surgery: final results of the SIOPEL-3HR study. *J Clin Oncol* 2010;28:2584–2590.
- 40 Czauderna P. Adult type vs childhood hepatocellular carcinoma—are they the same or different lesions? Biology, natural history, prognosis, and treatment. *Med Pediatr Oncol* 2002;39:519–523.
- 41 Llovet JM, Ricci S, Mazzaferro V, *et al*. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378–390.
- 42 Katzenstein HM, London WB, Douglass EC, *et al*. Treatment of unresectable and metastatic hepatoblastoma: a pediatric oncology group phase II study. *J Clin Oncol* 2002;20:3438–3444.
- 43 Kakar S, Chen X, Ho C, *et al*. Chromosomal changes in fibrolamellar hepatocellular carcinoma detected by array comparative genomic hybridization. *Modern pathol* 2009;22:134–141.

- 44 Venkatramani R, Wang L, Malvar J, *et al*. Tumor necrosis predicts survival following neo-adjuvant chemotherapy for hepatoblastoma. *Pediatr Blood Cancer* 2012;59:493–498.
- 45 Wellen JR, Anderson CD, Doyle M, *et al*. The role of liver transplantation for hepatic adenomatosis in the pediatric population: case report and review of the literature. *Pediatr Transpl* 2010;14:E16–E19.
- 46 Zucman-Rossi J, Jeannot E, Nhieu JT, *et al*. Genotype-phenotype correlation in hepatocellular adenoma: new classification and relationship with HCC. *Hepatology* 2006;43:515–524.
- 47 Bioulac-Sage P, Balabaud C, Bedossa P, *et al*. Pathological diagnosis of liver cell adenoma and focal nodular hyperplasia: Bordeaux update. *J Hepatol* 2007;46:521–527.
- 48 Lopez-Terrada D, Finegold MJ. Tumors of the liver, In: Suchy FJ(ed) *Liver Disease in Children*. Cambridge University Press: New York, NY, USA, 2012.
- 49 Christison-Lagay ER, Burrows PE, Alomari A, *et al*. Hepatic hemangiomas: subtype classification and development of a clinical practice algorithm and registry. *J Pediatr Surg* 2007;42:62–67 (Discussion 7–8).
- 50 Mo JQ, Dimashkieh HH, Bove KE. GLUT1 endothelial reactivity distinguishes hepatic infantile hemangioma from congenital hepatic vascular malformation with associated capillary proliferation. *Hum Pathol* 2004;35:200–209.
- 51 Han SJ, Tsai CC, Tsai HM, *et al*. Infantile hemangioendothelioma with a highly elevated serum alpha-fetoprotein level. *Hepatogastroenterology* 1998;45:459–461.
- 52 Available from: www.childrenshospital.org/cfapps/liverhem/index.cfm.
- 53 Ackermann O, Fabre M, Franchi S, *et al*. Widening spectrum of liver angiosarcoma in children. *J Pediatr Gastroenterol Nutr* 2011;53:615–619.
- 54 Eckardt MA, Chang VY, Nelson SD, *et al*. Not all hemangiomas are benign–epithelioid hemangioendothelioma: an aggressive vascular lesion in children. *Pediatr hematol oncol* 2011;28:622–624.
- 55 Levy M, Trivedi A, Zhang J, *et al*. Expression of glypican-3 in undifferentiated embryonal sarcoma and mesenchymal hamartoma of the liver. *Hum Pathol* 2012;43:695–701.
- 56 Bove KE, Blough RI, Soukup S. Third report of t(19q)(13.4) in mesenchymal hamartoma of liver with comments on link to embryonal sarcoma. *Pediatr Dev Pathol* 1998;1:438–442.
- 57 Alaggio R, Boldrini R, Di Venosa B, *et al*. Pediatric extra-renal rhabdoid tumors with unusual morphology: a diagnostic pitfall for small biopsies. *Pathol res prac* 2009;205:451–457.
- 58 Sainati L, Leszl A, Stella M, *et al*. Cytogenetic analysis of hepatoblastoma: hypothesis of cytogenetic evolution in such tumors and results of a multicentric study. *Cancer Genet Cytogenet* 1998;104:39–44.
- 59 Schneider NR, Cooley LD, Finegold MJ, *et al*. The first recurring chromosome translocation in hepatoblastoma: der(4)t(1;4)(q12;q34). *Genes Chromosomes Cancer* 1997;19:291–294.
- 60 Tomlinson GE, Douglass EC, Pollock BH, *et al*. Cytogenetic evaluation of a large series of hepatoblastomas: numerical abnormalities with recurring aberrations involving 1q12-q21. *Genes Chromosomes Cancer* 2005;44:177–184.
- 61 Giardiello FM, Petersen GM, Brensinger JD, *et al*. Hepatoblastoma and APC gene mutation in familial adenomatous polyposis. *Gut* 1996;39:867–869.
- 62 Koch A, Denkhaus D, Albrecht S, *et al*. Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. *Cancer Res* 1999;59:269–273.
- 63 Taniguchi K, Roberts LR, Aderca IN, *et al*. Mutational spectrum of beta-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas. *Oncogene* 2002;21:4863–4871.
- 64 Eichenmuller M, Gruner I, Hagl B, *et al*. Blocking the hedgehog pathway inhibits hepatoblastoma growth. *Hepatology* 2009;49:482–490.
- 65 Honda S, Arai Y, Haruta M, *et al*. Loss of imprinting of IGF2 correlates with hypermethylation of the H19 differentially methylated region in hepatoblastoma. *Br J Cancer* 2008a;99:1891–1899.
- 66 Lopez-Terrada D, Gunaratne PH, Adesina AM, *et al*. Histologic subtypes of hepatoblastoma are characterized by differential canonical Wnt and Notch pathway activation in DLK+ precursors. *Hum Pathol* 2009;40:783–794.
- 67 Nagai H, Naka T, Terada Y, *et al*. Hypermethylation associated with inactivation of the SOCS-1 gene, a JAK/STAT inhibitor, in human hepatoblastomas. *J Hum Genet* 2003;48:65–69.
- 68 von Schweinitz D, Fuchs J, Gluer S, *et al*. The occurrence of liver growth factor in hepatoblastoma. *Eur J Pediatr Surg* 1998;8:133–136.
- 69 Wagner F, Henningsen B, Lederer C, *et al*. Rapamycin blocks hepatoblastoma growth *in vitro* and *in vivo* implicating new treatment options in high-risk patients. *Eur J Cancer* 2012;48:2442–2450.
- 70 Adesina AM, Lopez-Terrada D, Wong KK, *et al*. Gene expression profiling reveals signatures characterizing histologic subtypes of hepatoblastoma and global deregulation in cell growth and survival pathways. *Hum Pathol* 2009;40:843–853.
- 71 Cairo S, Armengol C, De Reynies A, *et al*. Hepatic stem-like phenotype and interplay of Wnt/beta-catenin and Myc signaling in aggressive childhood liver cancer. *Cancer Cell* 2008;14:471–484.
- 72 Luo JH, Ren B, Keryanov S, *et al*. Transcriptomic and genomic analysis of human hepatocellular carcinomas and hepatoblastomas. *Hepatology* 2006;44:1012–1024.
- 73 Nagata T, Takahashi Y, Ishii Y, *et al*. Transcriptional profiling in hepatoblastomas using high-density oligonucleotide DNA array. *Cancer Genet Cytogenet* 2003;145:152–160.
- 74 Yamada S, Ohira M, Horie H, *et al*. Expression profiling and differential screening between hepatoblastomas and the corresponding normal livers: identification of high expression of the PLK1 oncogene as a poor-prognostic indicator of hepatoblastomas. *Oncogene* 2004;23:5901–5911.
- 75 Cairo S, Wang Y, de Reynies A, *et al*. Stem cell-like micro-RNA signature driven by Myc in aggressive liver cancer. *Proc Natl Acad Sci USA* 2010;107:20471–20476.
- 76 Malogolowkin MH, Katzenstein H, Krailo MD, *et al*. Intensified platinum therapy is an ineffective strategy for improving outcome in pediatric patients with advanced hepatoblastoma. *J Clin Oncol* 2006;24:2879–2884.
- 77 Perilongo G, Maibach R, Shafford E, *et al*. Cisplatin versus cisplatin plus doxorubicin for standard-risk hepatoblastoma. *N Engl J Med* 2009;361:1662–1670.

- 78 Finegold MJ, Lopez-Terrada DH, Bowen J, *et al*. Protocol for the examination of specimens from pediatric patients with hepatoblastoma. Arch Pathol Lab Med 2007;131:520–529.
- 79 Aronson DC, Schnater JM, Staalman CR, *et al*. Predictive value of the pretreatment extent of disease system in hepatoblastoma: results from the International Society of Pediatric Oncology Liver Tumor Study Group SIOPEL-1 study. J Clin Oncol 2005;23:1245–1252.
- 80 Warmann SW, Fuchs J. Drug resistance in hepatoblastoma. Curr Pharm Biotechnol 2007;8:93–97.