

Intra-tumor genetic heterogeneity in Wilms tumor samples

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<http://dx.doi.org/10.1590/1806-9282.65.12.1496>

SUMMARY

Childhood renal tumors account for ~7% of all childhood cancers, and most cases are embryonic Wilms' tumors (WT). Children with WT are usually treated by either COG or SIOP. The later treats the children using preoperative chemotherapy, but both have around 90% of overall survival in five years. WT is a genetically heterogeneous group with a low prevalence of known somatic alterations. Only around 30% of the cases present mutation in known genes, and there is a relatively high degree of intra-tumor genetic heterogeneity (ITGH). Besides potentially having an impact on the clinical outcome of patients, ITGH may interfere with the search for molecular markers that are prospectively being tested by COG and SIOP. In this review, we present the proposal of the current UMBRELLA SIOP Study 2017/Brazilian Renal Tumor Group that requires the multi-sampling collection of each tumor to better evaluate possible molecular markers, as well as to understand WT biology

KEYWORDS: Wilms tumor. Biomarkers. Genetic Heterogeneity

INTRODUCTION

Childhood renal tumors account for ~7% of all childhood cancers, and most cases are Wilms tumors (WT) or nephroblastomas (~90%), affecting one in 10,000 children under the age of fifteen¹. It is estimated there are 500 new cases of WT every year in Brasil, with a median age-adjusted incidence rate

of 9.5 cases per million². The peak incidence is between the ages of 2 and 3 years¹, but bilateral cases and those associated with congenital syndromes (5 to 10% of the cases) are diagnosed earlier³.

Non-WT renal tumors include clear cell sarcoma of the kidney (CCSK, 2-3%, 1% of the cases), with

DATE OF SUBMISSION: 04-Apr-2019

DATE OF ACCEPTANCE: 18-Jun-2019

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similar age presentation to WT, malignant rhabdoid tumor of the kidney (MRTK, 2-3%, 1% of the cases), which presents a peak incidence at 10 to 18 months, renal cell carcinoma (RCC, 1% of the cases), with a peak incidence among adolescents, benign congenital mesoblastic nephroma (CMF, 2-3% of the cases), and all other renal tumors, which include sarcoma and neuroectodermal tumor of the kidney, comprise 2-3% of the cases⁴. All renal tumors, but CMF, which is usually treated with surgery alone, have a poorer prognosis compared to WT. Due to its relatively higher incidence, this review will focus on WT.

Children with renal tumors are usually treated according to one of the two major clinical groups. The SIOP-RTSG (*Societe Internationale d'Oncologie Pediatric - Renal Tumor Study Group*) protocol recommends children be treated with pre-operative chemotherapy; and the COG (*National Wilms Tumor Study/Children's Oncology Group*) protocols advise children to be submitted to upfront surgery, with both reaching 90% of overall survival^{5,6}.

GENETIC EVENTS AND POTENTIAL PROGNOSTIC MARKERS FOR WILMS TUMORS

Most pediatric tumors present a short time that precedes the diagnosis, thus resulting in fewer evolutionary events compared to adult cancers⁷. Consistently, the mutational spectrum described in most pediatric solid tumors has been considerably small, including in WT. WT is a genetically heterogeneous group that presents a low prevalence of known somatic alterations and a relatively high degree of Intra-Tumor Genetic Heterogeneity (ITGH)^{8,9}. For instance, 30% of WT cases have a known mutated gene, with *WT1*, *CTNNB1*, and *AMER1* being the most prevalent^{10,11}. Moreover, alterations in microRNA processor genes (*DROSHA*, *DGCR8*, *DICER1*, and *TARBP2*) and *SIX1/2* were also found in up to 15% of WT¹²⁻¹⁵. The mutation spectrum of WT was properly discussed by other studies⁴⁻¹¹. For the purpose of this review, we considered of interest the somatic mutations in *TP53* found in ~70% of the diffuse anaplastic WT and gain of *MYCN* found in up to 4% of the cases. Diffuse anaplastic tumors comprise 5% of WT cases. They present an unstable genome, but tumors that also had *TP53* mutations or loss presented a higher number of copy number alterations^{14,16}. In diffuse anaplastic WT, mutation/loss

in *TP53* was suggested to be an independent poor prognostic factor¹⁶. *MYCN* gain was associated with anaplasia and with poorer relapse-free and overall survival, independently of tumor histology¹⁷. A gain of 1q is found in up to 30% of WT cases and was considered a potential prognostic biomarker regardless of the treatment protocol (COG or SIOP). A gain of 1q was associated with poorer event-free and overall survival, and, if validated, it could be used to select patients who were first treated with surgery or chemotherapy for more aggressive treatment¹⁸⁻²⁰.

Loss of heterozygosity (LOH) of both 1p and 16q were associated with lower event-free and overall survival in WT treated with surgery first^{21,22}. Following these findings, for the first time, the presence of molecular alterations was used to direct therapy in WT. COG intensified the treatment for stage III/IV WT, with loss of heterozygosity (LOH) of 16q, and 1p significant improving the event-free survival²³.

All potential biomarkers discussed here were studied in a single sample from each case without considering the existence of ITGH in WT.

Intra-tumor genetic heterogeneity in Wilms Tumor: the importance of prognostic markers

WT develops from primitive renal cells incapable of completing kidney differentiation, which results in a tumor that recapitulates nephrogenesis, with morphology, methylation, and gene expression similar to the fetal kidney²⁴⁻²⁸. WT is composed of varying proportions of three morphologically distinct cell types: undifferentiated blastemal cells, epithelial cells ordered into primitive structures, and stromal cells, which are related to the clinical behavior^{29,30}.

Intratumoral diversity is relatively common in chemotherapy-treated primary childhood cancers, even for WT that usually is described as presenting a relatively stable genome. Most WT subclones have low-frequency aberrations, but potential drivers may emerge as part of the ITGH, such as copy number neutral imbalance of 11p and trisomy 8, 1q gain, and 1p/16q loss⁹.

The gain of 1q is a promising biomarker for patients with WT stratification into risk groups, although it can be an early or late event. In the latter scenario, for prospective studies that underlie clinical trials, the assessment of the ITGH by multisampling the tumor for proper evaluation of this biomarker is of utmost importance. Based on an analysis of 20

cases of WT, it was estimated the need for at least three tumor samples for each case³¹.

Other alterations were characterized as ITGH in WT, such as *AMER1* (*WTX*)³², *DROSHA*, *SIX1*³³, and *TP53*³⁴. These studies are still in early stages, and definitive interpretation and conclusion will only be possible after studying large multi-sampled tumor cohorts.

Proposal of the current SIOP protocol/ Brazilian Renal Tumor Group

There is an increasing effort to identify prognostic molecular markers for patients with WT. The current approach to risk stratification has reached the limits of what can be achieved through combinations of clinical and pathological features. The biological mechanisms involved in tumor treatment response still need to be uncovered and associated with the current risk factors to further improve prediction of each child's risk of relapse. Retrospective studies from SIOP and COG pointed to alterations that are being explored prospectively in patients with WT. However, any potential prognostic markers require prospective validations that consider the underlying ITGH of each case.

The current protocol recommends the collection of three tumor samples at different sites of the tumor and the adjacent normal kidney by the pathologist responsible for the diagnosis at the time of surgery. The area of the research sample must be removed from the region immediately adjacent to the diagnostic sample (paraffin block), avoiding areas of

necrosis and hemorrhage (Figure 1A). It is extremely important to correctly identify each tube and record patient data to enable later association of experimental findings with the clinical-pathological characteristics. The tubes should be labeled as tumor (TW1, TW2, TW3, and so on) and kidney (NK1, NK2) samples for proper handling at the biomarker research stage. Note that the normal counterpart for comparison with WT is the renal cortex and not the kidney medulla. Samples may be used for DNA, RNA, and/or protein satisfactorily.

Samples should be collected immediately after surgery, frozen in liquid nitrogen, and sent to the Biobank of the institution for correct storage (Figure 2). For institutions without a Biobank, it is possible to ask for a tube with an appropriate reagent for tissue storage that keeps the tissue viable for further experiments. If this is the case, recommendations are: 1. the sample should be completely submerged by the reagent; 2. one of the dimensions of the tissue should be smaller than 0.5 cm to enable rapid stabilization of the molecules; 3. the approximate ideal ratio is 1:10, equivalent to 10 mg of tissue to 100 μ L of reagent. Each tube contains between 800 and 1000 μ L of reagent. Samples will be safe if stored at 37 °C for 24 hours; 15-25 °C for one week; 4 °C for six months; -20 or -80 °C indefinitely. These tubes are provided only by the project coordinator and should be requested to the SIOP-BRTG via SOBOPE (Figure 1B). In one year, the BRTG received eighteen cases from six institutions with at least three tumor and

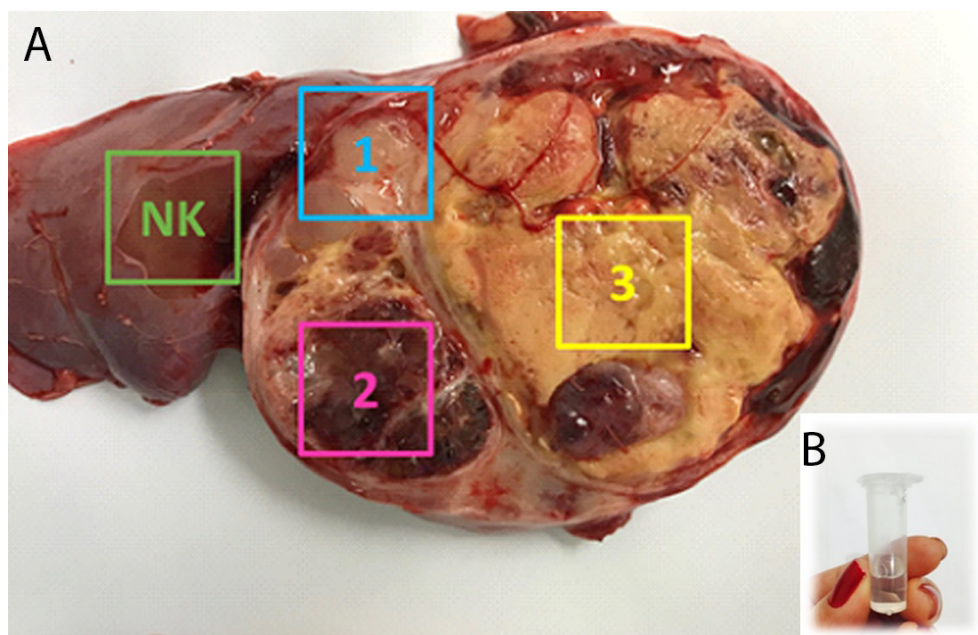


FIGURE 1. A. NEPHRECTOMY SPECIMEN. SAMPLES WERE COLLECTED FROM THREE TUMOR AREAS (1, 2, 3) AND ADJACENT NORMAL KIDNEY (NK) BY THE PATHOLOGIST. B – SCHEME FOR SAMPLE COLLECTION. TUBE TO KEEP THE FRESH TISSUE SAMPLE (800-1000 μ L OF REAGENT FOR 80 TO 100 MG OF TISSUE).

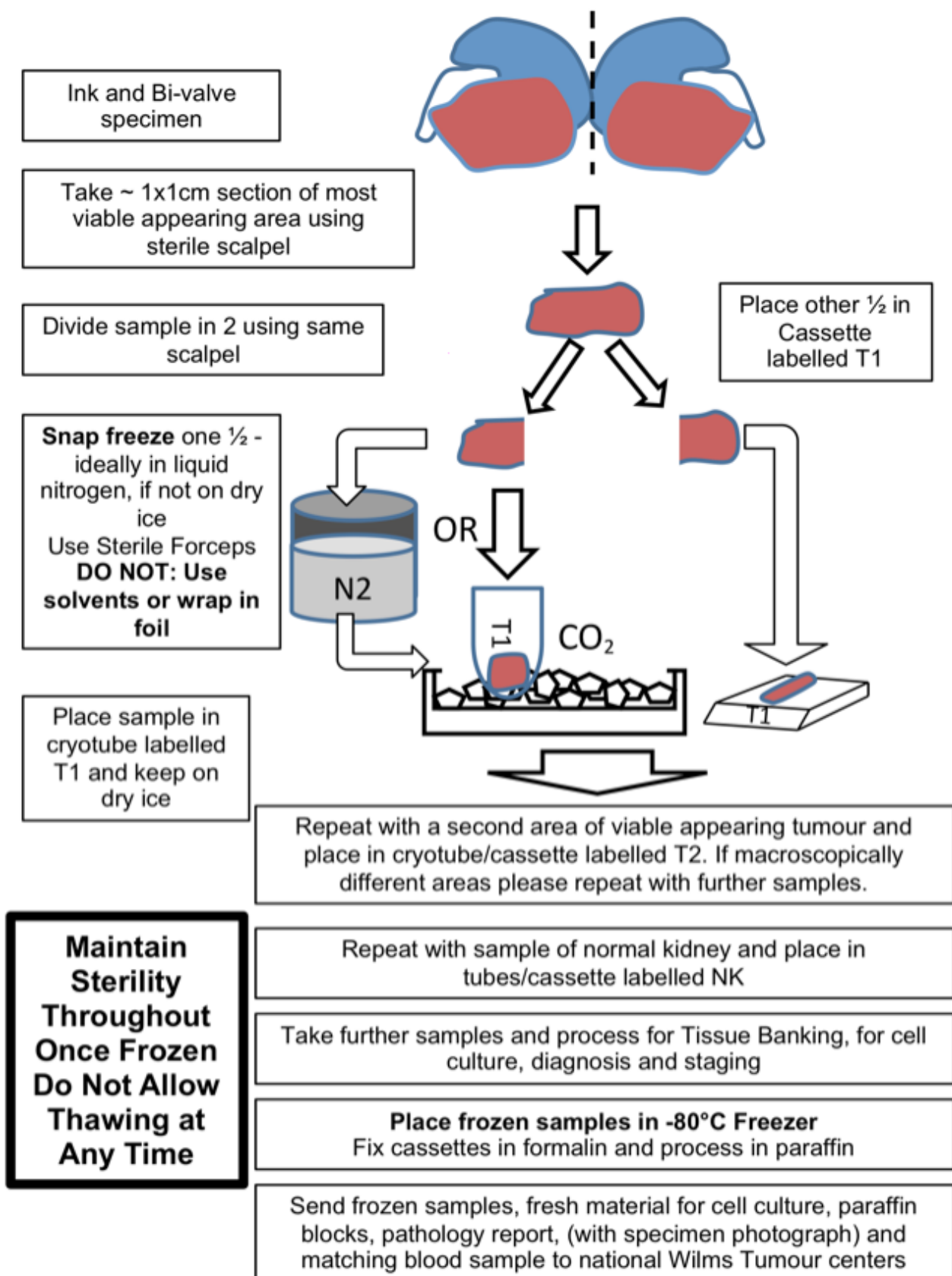


FIGURE 2. WORKFLOW FOR SUBMISSION OF TISSUE SAMPLES. SCHEME IS PART OF THE UMBRELLA SIOP-RTSG STUDY AND WAS KINDLY PROVIDED BY PROFESSOR KATHY PRITCHARD-JONES

one normal kidney samples from each case. All presented good quality for molecular analysis, pointing to the viability of this proposal.

These procedures are part of an attempt by the Brazilian Renal Tumors Group (BRTG) to promote research in renal tumors and to have active participation in molecular studies of international groups. Discussions regarding clinical, radiological, and pathological aspects of the patients are promptly discussed by email (tuwi_comite@googlegroups.com). More information is available at www.gbtr.com.br to increase participation in cooperative groups and improve diagnosis and treatment in such a heterogeneous country. All procedures were approved by the national ethical committee (CONEP 1.480.548; 2017).

Regarding WT pathology and molecular biology protocols, the BRTG complies with the UMBRELLA SIOP-RTSG 2016 updated guidelines, carefully reviewed by a consensus of pathology experts within the group, who also proposed to stimulate international collaboration with the harmonization of treatment protocols and research, including the standardization of specimen handling and improved collection of biological samples³⁵. These are of utmost impor-

tance to validate biomarkers such as *MYCN* amplification, 1q gain, and 17p loss. After all, the development and inclusion of true predictive biomarkers for pediatric patients with WT will only be possible by enabling large, international, high-quality databases and samples within cooperative studies.

CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgments

The authors are grateful to all the collaborators of the Brazilian Renal Tumor Group and to Maria Theresa de Sousa Accioly, Ph.D. and coordinator of the National Bank of tumors of INCA for supporting the storage of samples.

Author's contributions

All authors made substantial contributions to the development of the study, providing samples, drafting the manuscript with critical revision, and providing final approval of the version to be published.

RESUMO

Os tumores renais pediátricos correspondem a aproximadamente 7% de todos os tumores infantis, sendo o mais frequente o tumor de Wilms (TW). Crianças com TW são geralmente tratadas seguindo dois distintos protocolos terapêuticos (COG ou SIOP), sendo que no último, os pacientes recebem tratamento quimioterápico pré-operatório. Ambos apresentam sobrevida global em cinco anos em torno de 90%. TW é geneticamente heterogêneo, apresentando baixa prevalência de alterações somáticas conhecidas, com cerca de 30% dos casos apresentando mutações em genes conhecidos e um alto grau de heterogeneidade genética intratumoral (HGIT). Além de potencialmente ter um impacto sobre o desfecho clínico dos pacientes, a HGIT pode interferir na busca de marcadores moleculares que estão sendo testados prospectivamente pelos grupos COG e Siop. Nesta revisão, apresentamos a proposta do atual estudo Umbrella Siop 2017/Grupo de Tumores Renais Brasileiros (GTRB), que orienta a coleta de três diferentes regiões do tumor para melhor avaliar possíveis marcadores moleculares, bem como para compreender a biologia do TW.

PALAVRAS-CHAVE: Tumor de Wilms. Biomarcadores. Heterogeneidade genética.

REFERENCES

1. Stiller CA, Parkin DM. International variations in the incidence of childhood renal tumours. *Br J Cancer*. 1990;62(6):1026-30.
2. Camargo B, Oliveira Ferreira JM, Souza Reis R, Ferman S, Oliveira Santos M, Pombo-de-Oliveira MS. Socioeconomic status and the incidence of non-central nervous system childhood embryonic tumours in Brazil. *BMC Cancer*. 2011;5:11:160.
3. Dumoucel S, Gauthier-Villars M, Stoppa-Lyonnet D, Parisot P, Brisse H, Philippe-Chomette P, et al. Malformations, genetic abnormalities, and Wilms tumor. *Pediatr Blood Cancer*. 2014;61(1):140-4.
4. Brok J, Treger TD, Gooskens SL, van den Heuvel-Eibrink MM, Pritchard-Jones K. Biology and treatment of renal tumours in childhood. *Eur J Cancer*. 2016;68:179-95.
5. D'Angio GJ. The National Wilms Tumor Study: a 40 year perspective. *Lifetime Data Anal*. 2007;13(4):463-70.
6. SIOP Renal Tumour Study Group. Paediatric renal tumours: perspectives from the SIOP-RTSG. *Nat Rev Urol*. 2017;14(1):3-4.
7. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science*. 2013;339(6127):1546-58.
8. Gadd S, Huff V, Walz AL, Ooms AHAG, Armstrong AE, Gerhard DS, et al. A Children's Oncology Group and TARGET initiative exploring the genetic landscape of Wilms tumor. *Nat Genet*. 2017;49(10):1487-94.
9. Mengelbier LH, Karlsson J, Lindgren D, Valind A, Lilljebjörn H, Jansson C, et al. Intratumoral genome diversity parallels progression and predicts outcome in pediatric cancer. *Nat Commun*. 2015;6:6125.

10. Scott RH, Murray A, Baskcomb L, Turnbull C, Loveday C, Al-Saadi R, et al. Stratification of Wilms tumor by genetic and epigenetic analysis. *Oncotarget*. 2012;3(3):327-35.
11. Ruteshouser EC, Robinson SM, Huff V. Wilms tumor genetics: mutations in WT1, WTX, and CTNNB1 account for only about one-third of tumors. *Genes Chromosomes Cancer*. 2008;47(6):461-70.
12. Torrezan GT, Ferreira EN, Nakhata AM, Barros BD, Castro MT, Correa BR, et al. Recurrent somatic mutation in DROSHA induces microRNA profile changes in Wilms tumour. *Nat Commun*. 2014;9;5:4039.
13. Walz AL, Ooms A, Gadd S, Gerhard DS, Smith MA, Guidry Auvil JM, et al. Recurrent DGCR8, DROSHA, and SIX homeodomain mutations in favorable histology Wilms tumors. *Cancer Cell*. 2015;27(2):286-97.
14. Wegert J, Ishaque N, Vardapour R, Geörg C, Gu Z, Bieg M, et al. Mutations in the SIX1/2 pathway and the DROSHA/DGCR8 miRNA microprocessor complex underlie high-risk blastemal type Wilms tumors. *Cancer Cell*. 2015;27(2):298-311.
15. Rakheja D, Chen KS, Liu Y, Shukla AA, Schmid V, Chang TC, et al. Somatic mutations in DROSHA and DICER1 impair microRNA biogenesis through distinct mechanisms in Wilms tumours. *Nat Commun*. 2014;2:4802.
16. Maschietto M, Williams RD, Chagtai T, Popov SD, Sebire NJ, Vujanic G, et al. TP53 mutational status is a potential marker for risk stratification in Wilms tumour with diffuse anaplasia. *PLoS One*. 2014;9(10):e109924.
17. Williams RD, Chagtai T, Alcaide-German M, Apps J, Wegert J, Popov S, et al. Multiple mechanisms of MYCN dysregulation in Wilms tumour. *Oncotarget*. 2015;6(9):7232-43.
18. Segers H, van den Heuvel-Eibrink MM, Williams RD, van Tinteren H, Vujanic G, Pieters R, et al. Gain of 1q is a marker of poor prognosis in Wilms' tumors. *Genes Chromosomes Cancer*. 2013;52(11):1065-74.
19. Chagtai T, Zill C, Dainese L, Wegert J, Savola S, Popov S, et al. Gain of 1q as a prognostic biomarker in Wilms tumors (WTs) treated with preoperative chemotherapy in the International Society of Paediatric Oncology (SIOP) WT 2001 Trial: a SIOP Renal Tumours Biology Consortium Study. *J Clin Oncol*. 2016;34(26):3195-203.
20. Gratias EJ, Dome JS, Jennings LJ, Chi YY, Tian J, Anderson J, et al. Association of chromosome 1q gain with inferior survival in favorable-histology Wilms tumor: a report from the children's oncology group. *J Clin Oncol*. 2016;34(26):3189-94.
21. Grundy PE, Breslow NE, Li S, Perlman E, Beckwith JB, Ritchey ML, et al; National Wilms Tumor Study Group. Loss of heterozygosity for chromosomes 1p and 16q is an adverse prognostic factor in favorable-histology Wilms tumor: a report from the National Wilms Tumor Study Group. *J Clin Oncol*. 2005;23(29):7312-21.
22. Spreafico F, Gamba B, Mariani L, Collini P, D'Angelo P, Pession A, et al. AIEOP Wilms Tumor Working Group. Loss of heterozygosity analysis at different chromosome regions in Wilms tumor confirms 1p allelic loss as a marker of worse prognosis: a study from the Italian Association of Pediatric Hematology and Oncology. *J Urol*. 2013;189(1):260-6.
23. Dix DB, Fernandez CV, Chi Y-Y, Anderson JR, Mullen EA, Geller JJ, et al. Augmentation of therapy for favorable histology Wilms tumor combined with loss of heterozygosity of chromosomes 1p and 16q: a report from the Children's Oncology Group studies AREN0532 and AREN0533. *J Clin Oncol*. 2015;33:15.
24. Beckwith JB, Zuppan CE, Browning NG, Moksness J, Breslow NE. Histological analysis of aggressiveness and responsiveness in Wilms' tumor. *Med Pediatr Oncol*. 1996;27(5):422-8.
25. Maschietto M, Camargo B, Brentani H, Grundy P, Sredni ST, Torres C, et al. Molecular profiling of isolated histological components of Wilms tumor implicates a common role for the Wnt signaling pathway in kidney and tumor development. *Oncology*. 2008;75(1-2):81-91.
26. Charlton J, Williams RD, Sebire NJ, Popov S, Vujanic G, Chagtai T, et al. Comparative methylome analysis identifies new tumour subtypes and biomarkers for transformation of nephrogenic rests into Wilms tumour. *Genome Med*. 2015;7(1):11.
27. Fukuzawa R, Anaka MR, Morison IM, Reeve AE. The developmental programme for genesis of the entire kidney is recapitulated in Wilms tumour. *PLoS One*. 2017;12(10):e0186333.
28. Young MD, Mitchell TJ, Vieira Braga FA, Tran MGB, Stewart BJ, Ferdinand JR, et al. Single-cell transcriptomes from human kidneys reveal the cellular identity of renal tumors. *Science*. 2018;361(6402):594-9.
29. Perlman EJ. Pediatric renal tumors: practical updates for the pathologist. *Pediatr Dev Pathol*. 2005;8(3):320-38.
30. Rivera MN, Haber DA. Wilms' tumour: connecting tumorigenesis and organ development in the kidney. *Nat Rev Cancer*. 2005;5(9):699-712.
31. Cresswell GD, Apps JR, Chagtai T, Mifsud B, Bentley CC, Maschietto M, et al. Intra-tumor genetic heterogeneity in Wilms tumor: clonal evolution and clinical implications. *EBioMedicine*. 2016;9:120-9.
32. Wegert J, Wittmann S, Leuschner I, Geissinger E, Graf N, Gessler M. WTX inactivation is a frequent, but late event in Wilms tumors without apparent clinical impact. *Genes Chromosomes Cancer*. 2009;48(12):1102-11.
33. Spreafico F, Ciceri S, Gamba B, Torri F, Terenziani M, Collini P, et al. Chromosomal anomalies at 1q, 3, 16q, and mutations of SIX1 and DROSHA genes underlie Wilms tumor recurrences. *Oncotarget*. 2016;7(8):8908-15.
34. Wegert J, Vokuhl C, Ziegler B, Ernestus K, Leuschner I, Furtwängler R, et al. TP53 alterations in Wilms tumour represent progression events with strong intratumour heterogeneity that are closely linked but not limited to anaplasia. *J Pathol Clin Res*. 2017;3(4):234-48.
35. Vujančić GM, Gessler M, Ooms AHAG, Collini P, Coulomb-I'Hermine A, D'Hooghe E, et al; International Society of Paediatric Oncology–Renal Tumour Study Group (SIOP–RTSG). The UMBRELLA SIOP-RTSG 2016 Wilms tumour pathology and molecular biology protocol. *Nat Rev Urol*. 2018;15(11):693-701.

