



## Review

## Biology and treatment of renal tumours in childhood



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Received 18 March 2016; received in revised form 25 August 2016; accepted 1 September 2016

Available online 29 October 2016

## KEYWORDS

Renal tumour;  
Wilms' tumour;  
Clear cell sarcoma;  
Rhabdoid tumour;  
Renal cell carcinoma;  
Paediatric;  
Biology;  
Targeted therapy;  
SIOP-RTSG

**Abstract** In Europe, almost 1000 children are diagnosed with a malignant renal tumour each year. The vast majority of cases are nephroblastoma, also known as Wilms' tumour (WT). Most children are treated according to *Société Internationale d'Oncologie Pédiatrique* Renal Tumour Study Group (SIOP-RTSG) protocols with pre-operative chemotherapy, surgery, and post-operative treatment dependent on stage and histology. Overall survival approaches 90%, but a subgroup of WT, with high-risk histology and/or relapsed disease, still have a much poorer prognosis. Outcome is similarly poor for the rare non-WT, particularly for malignant rhabdoid tumour of the kidney, metastatic clear cell sarcoma of the kidney (CCSK), and metastatic renal cell carcinoma (RCC).

Improving outcome and long-term quality of life requires more accurate risk stratification through biological insights. Biomarkers are also needed to signpost potential targeted therapies for high-risk subgroups. Our understanding of Wilms' tumourigenesis is evolving and several signalling pathways, microRNA processing and epigenetics are now known to play pivotal roles. Most rhabdoid tumours display somatic and/or germline mutations in the *SMARCB1* gene, whereas CCSK and paediatric RCC reveal a more varied genetic basis, including characteristic translocations. Conducting early-phase trials of targeted therapies is challenging due to the scarcity of patients with refractory or relapsed disease, the rapid progression of relapse and the genetic heterogeneity of the tumours with a low prevalence of individual somatic mutations. A further consideration in improving population survival rates is the geographical variation in outcomes across Europe.

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This review provides a comprehensive overview of the current biological knowledge of childhood renal tumours alongside the progress achieved through international collaboration. Ongoing collaboration is needed to ensure consistency of outcomes through standardised diagnostics and treatment and incorporation of biomarker research. Together, these objectives constitute the rationale for the forthcoming SIOP-RTSG ‘UMBRELLA’ study.

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## 1. Introduction

Childhood renal tumours account for around 7% of all childhood cancers. The majority of cases (90%) are Wilms’ tumour (WT or nephroblastoma), with an annual incidence of approximately 1 in 100,000 children [1]. Thus, a large European country, such as Germany, will experience around 100 new cases a year, whereas a small country like Denmark diagnose less than 10 cases annually.

The median age at diagnosis of WT is 3 years but bilateral cases and those associated with congenital syndromes occur earlier. The most common presentation is that of an abdominal mass or swelling, and children are usually otherwise clinically well [2]. Other symptoms include abdominal pain, haematuria, fever, and symptoms related to hypertension. About 10% of WT have haematogenous spread, most commonly to the lungs (85%), liver (10%) and only very rarely to the bones and brain [1].

WT may occur as a part of a genetic predisposition syndrome in 5–10% of cases. The more common phenotypes include WAGR (WT, aniridia, genitourinary anomalies, and mental retardation), Denys–Drash syndrome, Beckwith–Wiedemann syndrome, asymmetric overgrowth, or family history of WT [3]. If predisposition is suspected prior to the diagnosis of WT, the tumour may be detected through a screening programme.

The most frequently occurring non-Wilms’ renal tumours (non-WT) include clear cell sarcoma of the kidney (CCSK) with an identical age distribution to WT, malignant rhabdoid tumour of the kidney (MRTK) with a peak incidence between the age of 10–18 months and renal cell carcinoma (RCC), which usually occurs in adolescence. Altogether, CCSK and MRTK comprise about 3–5% of all primary renal tumours in children, whereas RCC accounts for around 1% (Fig. 1) [4]. Other types of malignant renal tumours, such as anaplastic sarcoma and primitive neuroectodermal tumour of the kidney, are extremely rare. Overall non-WT have a poorer outcome than WT. Relatively benign renal tumours, such as congenital mesoblastic nephroma, are mainly diagnosed in newborns or during foetal anomaly scanning, and cure can usually be achieved with surgery alone.

There are two different approaches to the initial management of renal tumours in childhood. Most children in Europe are treated with pre-operative chemotherapy, according to the *Société Internationale d’Oncologie Pédiatrique* Renal Tumour Study Group (SIOP-RTSG) protocols. In North America, patients are treated with upfront surgery prior to administration of chemotherapy, as per the National Wilms’ Tumour Study/Children’s Oncology Group (COG) protocols. Although the SIOP and COG strategies differ in their upfront treatment approach, they have a similar overall survival (OS) of nearly 90% [5,6].

Despite the excellent prognosis for most children with WT, just under 15% of patients will relapse, usually within 2 years of diagnosis [5]. Furthermore, a proportion of patients will experience severe early and late treatment-related adverse events, e.g. cardiotoxicity secondary to doxorubicin (DOX) or radiotherapy-induced organ dysfunction, musculoskeletal abnormalities, infertility and secondary malignancies [7,8]. The current aims of treatment optimisation are to standardise diagnosis, to improve risk stratification, to minimise side-effects of treatment and to improve relapse monitoring according to clinical, molecular, histopathological and imaging data.

The aim of this review is to provide the clinician with an overview of tumour biology, current treatment and research into more effective therapies for subgroups of paediatric renal tumours. Focus will be on WT, due to its relatively higher incidence, but other renal tumours will be discussed within the context of the SIOP-RTSG strategy.

## 2. Biology of sporadic WT and predisposition syndromes

### 2.1. Genetics

Despite our incomplete understanding of the pathogenesis of WT, there is increasing evidence that several signalling pathways, microRNA processing, and epigenetics all play pivotal roles (Table 1). In general, WT represent a genetically heterogeneous group, displaying a low prevalence of known somatic alterations and a high degree of intra-tumoural heterogeneity [9].

The first WT-related gene to be characterised was *WT1*, a zinc finger DNA-binding transcription factor,

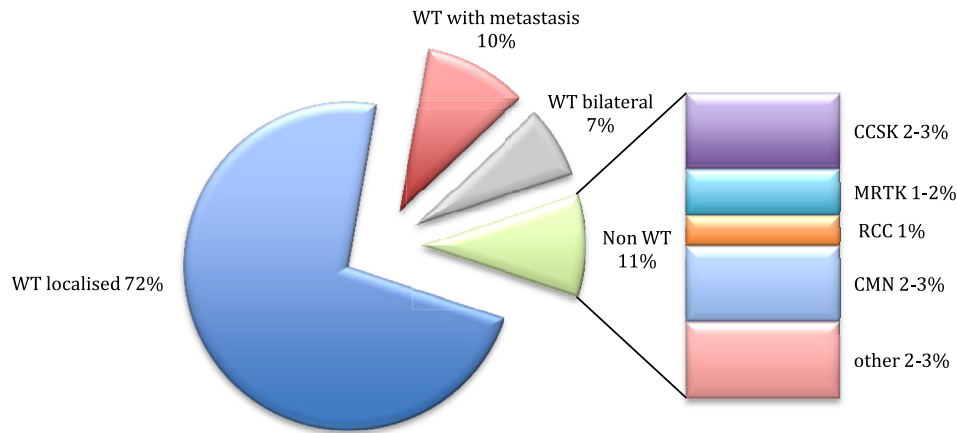


Fig. 1. Distribution of renal tumours in children registered in the SIOP-RTSG WT 2001 trial and study. Note that MRTK and RCC are underrepresented in this clinical database, compared to population-based cancer registration [165]. CCSK, clear cell sarcoma of the kidney; CMN, congenital mesoblastic nephroma; MRTK, malignant rhabdoid tumour of the kidney; RCC, renal cell carcinoma; WT, Wilms’ tumour.

Table 1  
Most frequently identified somatic changes in childhood renal tumours.

Genetics	Tumour	Approximate incidence	Clinicopathological associations	References
<i>WT1</i> (11p13)	WT	10–20%	Early event, found in ILNR; associated with stromal histology	[11,15]
<i>CTNNB1</i> (3p21)	WT	15%	Late event, not in NR	[16]
<i>MLL1</i> (19p13)	WT	4% in one series	Early event, found in ILNR; younger age	[18]
<i>WTX</i> (Xq11)	WT	15–20%	No clinicopathological associations	[19,21]
<i>IGF2</i> (11p15)	WT	69%	Early event, found in PLNR; associated with epithelial/blastemal histology	[14,38]
Loss of 1p, 16q	WT	1p 11%; 16q 17%; 1p/16q 5%	Combined LOH associated with reduced EFS and OS	[6]
Gain of 1q	WT	27%	Reduced EFS, possible reduced OS	[106,107,108]
<i>TP53</i> (17p13)	WT	70% of anaplastic tumours	Reduced EFS and OS; rarely found in tumours without diffuse anaplasia	[24,27]
<i>MYCN</i> (2p24)	WT	13%	Reduced EFS, OS; associated with anaplastic histology	[31]
miRNAPG: <i>DROSHA</i> , <i>DGCR8</i> , <i>DICER1</i> , <i>TARBP2</i> , <i>DIS3L2</i> and <i>XPO5</i>	WT	18% of SIOP high-risk blastema tumours 15% pre-therapy FHWT	Found in PLNR in FHWT; <i>DGCR8</i> has a female bias (88% of cases) reduced EFS, OS when found in combination with <i>SIX1/2</i> mutations	[25,32,42]
<i>SIX1</i> (14q23), <i>SIX2</i> (2p21)	WT	18% of SIOP high-risk blastema tumours 7% pre-therapy FHWT	Found in PLNR in FHWT; reduced EFS and OS when found in combination with <i>miRNAPG</i> mutations	[25,32]
<i>SMARCA4</i> (19p13)	WT ATRT	4.5% in one series 1 case report	Not known	[42,69]
<i>SMARCB1</i> (22q11)	MRTK ATRT	95%	Mutation found in almost all patients	[64,65]
<i>YWHAE-NUTM2</i> t(10;17) (q22;p13)	CCSK	12%	No clinicopathological associations	[54,58]
<i>ITD BCOR</i> (Xp11)	CCSK	85–100%	Not known	[59,60]
<i>TFE3</i> (Xp11) to <i>ASPL</i> (17q25), <i>PRCC</i> (1q21), <i>PSF</i> (1p34), <i>NonO</i> (Xq12), and <i>CLTC</i> (17q23)	RCC	20–40%	Translocation-type RCC	[72]
<i>TFEB</i> t(6;11) (p21;q12)	RCC	Case reports	Translocation-type RCC	[73]

ATRT, atypical teratoid/rhabdoid tumour; CCSK, clear cell sarcoma of the kidney; EFS, event-free survival; FHWT, favourable histology Wilms’ Tumour; ILNR, intralobar nephrogenic rests; LOH, loss of heterozygosity; OS, overall survival; PLNR, perilobar nephrogenic rests; PLNR, perilobar nephrogenic rests; RCC, renal cell carcinoma; SIOP, Société Internationale d’Oncologie Pédiatrique; WT, Wilms’ tumour.

with a vital role in nephrogenesis and glomerular function in the mature kidney [10–12]. Germline *WT1* mutations lead to a range of genitourinary malformations and underlie tumour development in both WAGR and Denys–Drash syndromes [13]. Approximately 5–20% of sporadic tumours also exhibit somatic *WT1* loss, with incidence dependent on patient ethnicity [14]. Mutations in *WT1* are also found in precursor lesions known as intralobar nephrogenic rests (ILNR), suggesting that they are early events in tumourigenesis [15].

*WT1* negatively regulates the WNT pathway and mutations in the *CTNNB1* gene, coding for the crucial protein beta-catenin, often occur alongside *WT1* loss [16,17]. Recently, somatic mutations in the *MLLT1* gene were identified in 4% of WT, frequently accompanied by *CTNNB1* mutations and WNT pathway activation [18]. *MLLT1* plays a critical role in transcriptional regulation during early renal developmental and patients with the mutation present at a younger age with a high prevalence of ILNR.

Beta-catenin is targeted for degradation by the ‘destruction complex’ which includes the WTX (*AMER1*) protein, this is itself inactivated in 18% of WTs [19–21]. The role of somatic *WTX* mutations in tumour development is unclear and germline mutations, found in sclerosing skeletal dysplasia, confer no increased risk of WT [22]. *WTX* interacts with the tumour suppressor protein p53, enhancing its role in cell cycle arrest and apoptosis [23].

Somatic mutations in *TP53* are only found in approximately 70% of the rare high-risk group of anaplastic cases [24]. These tumours have a characteristically unstable genome with multiple copy number aberrations and chromothripsis [25]. In addition to the classical role of p53 as a tumour suppressor protein, gain-of-function mutations are now thought to drive cell migration and invasion [26]. In WT, mutations in *TP53* are an independent indicator of poor prognosis [27]. Another protein thought to hold tumour suppressor function is the RE1 Silencing Transcription Factor (*REST*) transcription factor, and inactivating mutations have been identified in both sporadic and familial WT [28]. *REST* is a transcriptional repressor that is essential for embryonic development and truncations in the protein occur in several cancers [29].

Regarding driver genes, gain of the proto-oncogene *MYCN* is associated with poor outcome in several childhood embryonal cancers including WT, neuroblastoma, medulloblastoma and rhabdomyosarcoma [30]. Gain of *MYCN* is predominantly a somatic event but germline aberrations have also been reported [31]. *SIX1* and *SIX2* were also recently identified as other prognostically relevant candidate oncogenes, with somatic mutations found in both low- and high-risk histological subtypes [25,32]. These transcription factors are known to be involved in nephron development, with

*SIX2* maintaining mesenchyme progenitor cells in an undifferentiated blastema state and *SIX1*-knockout mice displaying renal agenesis [33,34].

## 2.2. Epigenetics

Disruption of epigenetic processes, such as DNA methylation and histone modification, is now known to contribute to the development and progression of many childhood solid tumours including WT [35,36].

Through analysis of patients with Beckwith–Wiedemann and related overgrowth syndromes, aberrant imprinting at 11p15 was implicated in WT development. The two principal abnormalities at this locus are paternal uniparental disomy and maternal *H19* epimutation, both resulting in activation of the insulin-like growth factor (IGF) signalling pathway [37]. This is the most common pathway that is deregulated in WT, with somatic aberrations found in 70% of sporadic tumours, and is considered to occur early in tumour development [14,38]. These WT are a clinically distinct group from those without the mutation and are associated with blastema/epithelial histology and perilobar nephrogenic rests [39]. The IGF pathway is involved in cell proliferation, inhibition of apoptosis and protein synthesis, via activation of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (P13K/Akt/mTOR) and ras–raf–mitogen-activated protein kinases (MAPK) signalling [40].

Many other changes to the epigenome have been identified in WT. Whole-exome sequencing has demonstrated mutations in the chromatin remodelers *SMARCA4* and *ARID1A*, previously identified in childhood medulloblastoma [41,42]. Analysis of methylation profiles shows significant variation between normal kidney, nephrogenic rests and tumour [43]. In addition, promoters for *SIX2* and *WT1* are hypomethylated in WT and foetal kidneys; in contrast, hypermethylation of these sequences is described in mature kidneys [44]. *WT1* itself can directly affect methylation through transcriptional regulation of the enzymes DNA methyltransferase 3A and DNA methyltransferase 1 [45,46]. *WT1* also recruits Polycomb proteins to reduce Pax2 expression, a transcriptional regulator that is critical for urogenital development and mesenchymal-to-epithelial transition (MET) [47]. Polycomb genes (*EZH2*, *BMI-1* and *SUZ12*) are upregulated in WT xenografts and reduced when tumour cells undergo differentiation, suggesting that they may maintain malignant renal progenitor cells [44].

## 2.3. MicroRNA processing pathway

MicroRNAs (miRNAs) are small non-coding RNAs involved in post-transcriptional gene silencing [48]. Mutations in several miRNA-processing genes (*DROSHA*, *DICER1*, *DGCR8*, *XPO5* and *TARBP2*) have

been identified in sporadic WT, both in chemotherapy-naive tumours and post chemotherapy [25,32,42]. *DROSHA* and *DICER1* mutations reduce expression of the tumour-suppressor Let7 family and lead to failure of epithelial differentiation [42]. Disruption of miRNA biogenesis through germline mutations in *DIS3L2* underlies Perlman syndrome, a rare congenital overgrowth syndrome with susceptibility to WT [49]. In addition to tumour development, specific miRNAs can predict response to chemotherapy and are associated with metastasis, suggesting a role in disease progression [50,51]. Several groups have explored their use as a diagnostic biomarker, demonstrating that serum miRNA profiles can reliably differentiate WT from other solid tumours and healthy controls [52,53].

### 3. Biology of non-WT

#### 3.1. Clear cell sarcoma of the kidney

CCSK is not associated with genetic predisposition syndromes and familial CCSK cases have not been reported. One of the recurring genetic changes is the translocation t(10;17) (q22;p13), occurring in about 12% of tumours [54]. This involves the fusion of *YWHAE* and *NUTM2B/E* genes on chromosome 17 and 10, respectively. The *YWHAE* gene encodes an epsilon protein, which modulates phosphoserine-containing proteins and plays a role in various signal transduction pathways including P13K/Akt and MAPK [55–57]. This translocation does not appear to be correlated with either tumour characteristics or patient outcome [58].

Recently, recurrent internal tandem duplications (ITD) of the X-linked BCL-6 co-repressor (*BCOR*) gene have been described and are found to be mutually exclusive to t(10;17) (q22;p13) [59,60]. Until recently, all reported CCSK patients without t(10;17) (q22;p13) were thought to harbour the *BCOR* ITD and vice versa, but a subset of tumours have been identified with neither mutation [61]. Finally, although CCSK is a genomically stable tumour, over-expression of several genes has been reported, including neural markers (e.g. nerve growth factor receptor) and genes involved in both the Sonic hedgehog pathway and the P13K/Akt cell proliferation pathway [62,63].

#### 3.2. Malignant rhabdoid tumour of the kidney

More than 95% of rhabdoid tumours have bi-allelic inactivating mutations of *SMARCB1*. Up to 35% of patients also have a germline mutation in one allele of *SMARCB1*, which is an additional adverse prognostic indicator [64–68]. *SMARCA4* mutations have been identified in a smaller subset of patients with the closely related atypical teratoid/rhabdoid tumour of the brain [69]. *SMARCB1* encodes a member of the SWItch/

Sucrose Non-Fermentable (SWI/SNF) chromatin remodelling complex, which regulates transcription of specific targets. Germline analysis is recommended for individuals of all ages with rhabdoid tumours and prenatal diagnosis can be performed in families with a known *SMARCB1* alteration. Surveillance guidelines for patients with a germline mutation have been developed [70]. Gene expression studies of rhabdoid tumour have identified multiple other genes and pathways with altered expression [71].

#### 3.3. Renal cell carcinoma

In children, translocation RCC is the most common subtype of RCC and is characterised by translocations involving the *transcription factor E3* gene (*TFE3*) on chromosome Xp11. This gene can fuse to several partners including *ASPL* (17q25), *PRCC* (1q21), *PSF* (1p34), *NonO* (Xq12), and *CLTC* (17q23) [72]. Another less-common translocation is t(6;11) (p21;q12) involving a fusion of *Alpha* (11q12) and *transcription factor EB* (*TFEB*) (6p21) that leads to overexpression of *TFEB* [73]. Of interest, 15% of patients with translocation RCC have previously been treated with chemotherapy [74].

Several genetic syndromes are associated with a predisposition to RCC. The best described is von Hippel-Lindau (VHL), caused by mutations or deletions in the *VHL* gene. *VHL* is a tumour suppressor gene that regulates the level of the hypoxia-inducible factor family of transcription factors and is involved in many cellular processes that are dysregulated in human cancer [75]. Affected individuals are mainly susceptible to clear cell type RCC. Tumours usually develop in adulthood, only rarely in childhood, and annual screening is recommended [70].

Tuberous sclerosis (TS) is another rare genetic syndrome that carries an increased risk of both RCC of the clear cell type and angiomyolipoma. The syndrome is caused by mutations in the *TSC1* and *TSC2* genes, which encode key regulators of the mTOR pathway [76]. Mutations in the *FLCN* gene, encoding the protein folliculin which interacts with the mTOR pathway, underlie the Birt–Hogg–Dubé syndrome [77]. Individuals with this syndrome are at risk of developing hybrid oncocytoma/chromophobe RCC. Mutations in the *MET* oncogene, encoding the hepatocyte growth factor receptor, lead to hereditary papillary RCC [78]. Children with germline mutations of two tricarboxylic acid (Krebs) cycle genes, fumarate hydratase (FH) and succinate dehydrogenase (*SDH*), are also susceptible to RCC [79].

## 4. Current models of tumours

#### 4.1. Genetically modified mouse models

Much effort has been directed towards preclinical models to further characterise Wilms' tumourigenesis

and to guide drug discovery. The identification of *WT1* in 1990 led to the development of early *WT1*-knockout mouse models [80]. Mice with homozygous loss displayed complete renal agenesis, dying prematurely, whilst those with a heterozygous genotype did not develop tumours. The first sustainable WT mouse model was developed in 2011 with *WT1* loss, leading to failure of MET of the metanephric blastema, and upregulation of *IGF2* resulting in arrested differentiation and cellular proliferation [81]. A second murine model arose from the finding that *Lin28* overexpression during kidney development prevents the final stage of differentiation, leading to WT formation in mice [82]. *Lin28* is an RNA-binding protein that suppresses *Let-7* miRNA processing and has also been implicated in the development of neuroblastoma and type II germ cell tumours [83,84].

#### 4.2. Cell lines

Development of cell lines with a sustained life span that are representative of the triphasic histological pattern of Wilms has been challenging, with many attempts found to represent other tumour types. The WiT49 anaplastic cell line, from a xenograft of a human WT lung metastasis, is stable with biphasic histology. It carries a *TP53* mutation, overexpresses *IGF2* and has wild-type *WT1*, with a high tumour occurrence rate when transplanted into recipient mice [85]. The cell line has been used to demonstrate that overexpression of *SIX2* shifts WNT/ $\beta$ -catenin signalling away from differentiation and towards stem cell survival and that the sphingosine-1-phosphate (S1P) pathway stimulates cell migration and invasion [86,87]. Initial testing of novel compounds has been carried out on WT and rhabdoid cell lines, predominantly by the paediatric preclinical testing programme, a North American initiative that evaluates novel compounds in solid tumour and leukaemia *in vivo* models. Encouraging results have recently been reported for WT with inhibitors of JAK1/2, topoisomerase II and exportin 1 [88–90]. The exportin 1 inhibitor selinexor demonstrated additional cytotoxic activity with rhabdoid cell lines, although all rhabdoid xenografts had progressive disease [90]. Unfortunately, for CCSK, it has not yet been feasible to grow cell lines.

#### 4.3. Novel models

Organoids are 3D adult organ-derived epithelial structures that contain self-renewing and organ-specific stem or progenitor cells, as well as differentiated cells [91]. Organoid cultures have proven to be of value for basic research, for the study of healthy tissue homeostasis and biology of disease. Kidney organoid systems are currently being developed for functional validation studies and preclinical drug testing of paediatric renal tumours [92].

In addition, the zebrafish has emerged as an excellent model for studies of vertebrate biology. External development and optical transparency during embryogenesis allow for visual analysis of early developmental processes, and high fecundity and short-generation time facilitate genetic analysis. The organisms have been used to study the effect of WT-related gene knockdowns on nephrogenesis and have confirmed the essential roles of *WT1* and *SIX2* [93,94]. More recently, zebrafish have provided insight into many human tumours and their role as a WT model remains promising [95].

### 5. Upcoming European SIOP-RTSG clinical study

For more than a decade, the SIOP-RTSG WT-2001 has been the main clinical trial and study enrolling children with renal tumours in Europe. This randomised clinical trial (RCT) has further optimised risk-stratified chemotherapy to omit DOX in stage II/III, intermediate-risk histology WT. It also highlighted the need to incorporate biomarker and imaging research into a standardised diagnostic and treatment protocol, the SIOP-RTSG ‘UMBRELLA 2016’ study [96]. The main research objective of this upcoming study is to understand the prognostic impact of somatic genetic biomarkers (including 1q gain, *TP53* mutation and *MYCN* aberrations). Another aim is to optimise the definition of high-risk ‘blastemal type’ WT by quantifying the volume of blastemal components that survives pre-operative chemotherapy and determining the correlation between absolute blastemal volume, biomarkers and clinical outcome [97]. A related aim is to identify molecular targets for novel therapeutic approaches, particularly for the high-risk groups. Central pathology review will be expanded across Europe in order to standardise diagnostics. Likewise, centralised radiology review will be introduced, with a focus on better definition of computed tomography (CT)-identified lung lesions and metastatic response. Expanding the number of countries and centres that participate is fundamental to collect sufficient biological material and clinicopathological data.

### 6. Management of WT

#### 6.1. Treatment

According to the SIOP-RTSG strategy, all patients with WT aged over 6 months receive pre-operative chemotherapy with actinomycin D (ACT-D) and vincristine (VCR). DOX is added in metastatic cases [5]. Pre-operative chemotherapy reduces the risk of tumour rupture thereby downstaging the tumour and decreasing the overall burden of therapy [98]. Radical nephrectomy is the current standard surgical treatment for children with unilateral WT. For bilateral tumours or children at high risk of bilateral disease, nephron sparing surgery

(NSS) should be attempted if feasible. NSS for unilateral disease, in order to preserve renal function without compromising oncological risk, may play a role for carefully selected patients in the future and guidelines have been defined in consensus with the surgical experts of the SIOP-RTSG group [96,99].

Following surgery, the histopathological features of the tumour stratify patients into three prognostic groups: low risk, intermediate risk and high risk (Table 2). The intensity of treatment is directed according to these prognostic groups, based on previous studies demonstrating the association between histopathology and survival [97]. Post-operative chemotherapy and the need for radiotherapy are further dictated by tumour stage (Table 3 and Supplementary Fig. 1).

Survival rate for WT is nearly 90%. Nevertheless, approximately 10% of patients with intermediate-risk histology and up to 25% of patients with high-risk tumours will relapse (Table 4) [5,100]. OS amongst relapsed patients is around 50% but with a large variance depending on the initial treatment, relapse site and tumour histology. These parameters guide future relapse treatment into three risk categories (standard, high and very high risk), where high-dose melphalan and autologous stem cell rescue are recommended as consolidation in both the high- and very high-risk groups [101]. Relapse treatment, in contrast to first line treatment, is not based on evidence from RCTs, but solely on prospective single-arm studies and, in very high-risk relapse, on case series [102–104].

6.2. Biomarkers

Currently, there is an increased focus on identifying better prognostic molecular markers for WT. Recommendations

of potential molecular markers in other tumour types are mushrooming due to the low cost, speed and ease of current genetic sequencing methods. Any potential prognostic indicators require prospective validation and assessment of intra-tumoural heterogeneity in a suitably powered cohort of patients. Furthermore, even if a biological marker is independently found to be significantly associated with clinical outcomes, the optimal adjustment of treatment in subgroups of patients with and without such markers is unknown.

Recently, the COG group assessed intensified (the addition of etoposide [ETO]/cyclophosphamide [CYC] to VCR/ACT-D and DOX) treatment for stage III/IV WT with loss of heterozygosity (LOH) of 16q and 1p (~5% of WT), which is associated with reduced OS [6,105]. Compared with a historical control group, they found that the new 5 drug regimen significantly improved event-free survival (EFS) and the intensified treatment is likely to continue as standard for favourable histology WT displaying LOH of 16q and 1p. Treatment was likewise intensified for stage I and II patients, with DOX added to VCR/ACT-D, though this did not significantly affect outcome. This is the first group to use molecular biomarkers to direct therapy in WT. A more common cytogenetic abnormality is gain of 1q, which is present in about 30% of tumours and associated with worse EFS and possible worse OS [106–108]. Likewise, MYCN gain and 17p loss (the TP53 locus), limited to anaplastic WT, may independently predict poor survival [27,31].

6.3. Late effects

Although associated with fewer late effects compared to other solid malignant tumours, WT survivors still reveal

Table 2

Comparison between histological subtyping and risk classification of WT according to the SIOP and COG treatment approaches, with % of patients in each subgroup.

Histopathology Risk Group	SIOP		Histopathology Risk Group	COG					
	Histological subtype post preoperative chemotherapy <sup>a</sup>			Histological subtype post immediate nephrectomy					
	L%	M%		L%	M%				
LOW	4	10	Completely necrotic Cystic partially differentiated	3 1	10 0	FAVOURABLE	90	80	No evidence of anaplasia
INTERMEDIATE	80	65	Regressive Stromal Epithelial Mixed Focal anaplasia	32 12 7 27 2	44 6 3 10 2				
HIGH	13	13	Diffuse anaplasia Blastemal	4 9	8 5				

L = local; M = metastatic

<sup>a</sup> 3% of localised and 12% of metastatic tumours were not classified

Source: SIOP WT 2001 database for all patients with stage I–IV WT, 5731 patients were registered from 2001 to 2015. Data provided by Harm van Tinteren, trial statistician. COG data from Ref. [166].

Table 3

Comparison between staging classification for WT according to the SIOP and COG approaches.

Stage	SIOP	COG
I	Tumour limited to kidney or surrounded with fibrous pseudocapsule and completely resected. Intrarenal vessel involvement may be present, no involvement of renal sinus vessels. Presence of necrotic tumour in the renal sinus or peri-renal fat does not upstage to stage II providing it does not reach the resection margins. Percutaneous cutting needle biopsy is allowed.	Tumour limited to kidney with intact renal capsule and completely resected with no evidence of the tumour at or beyond the margins of resection. Intrarenal vessel involvement may be present, no involvement of renal sinus vessels. No biopsy has been performed.
II	Tumour extension beyond kidney or renal pseudocapsule but completely resected. Infiltration of renal sinus and/or blood and lymphatic vessels outside renal parenchyma but completely resected. Local invasion of adjacent structures or extension into the vena cava is allowed providing resection is performed en bloc and there is no evidence of tumour at or beyond the resection margins.	Tumour extension beyond kidney or penetration of renal capsule but completely resected. Local invasion of adjacent structures or extension into the vena cava is allowed providing resection is performed en bloc and there is no evidence of tumour at or beyond the resection margins. Absence of tumour rupture or spillage, even confined to the flank. No biopsy has been performed.
III	Any of the following reasons, either individually or collectively, assign a tumour to stage III: (1) tumour extends to or beyond resection; margins microscopically or there is macroscopic incomplete excision, (2) positive abdominal lymph nodes; (3) tumour rupture before or intra-operatively including diffuse peritoneal contamination by the tumour or where peritoneal implants are present; (4) piecemeal removal of intravascular tumour thrombus; (5) open biopsy prior to pre-operative chemotherapy or surgery.	Any of the following reasons, either individually or collectively, assign a tumour to stage III: (1) tumour extends to or beyond resection margins microscopically or there is macroscopic incomplete excision; (2) positive abdominal lymph nodes; (3) tumour rupture before or intra-operatively including spillage confined to the flank or diffuse peritoneal contamination by the tumour or where peritoneal implants are present, (4) piecemeal removal of intravascular tumour thrombus; (5) any biopsy is performed prior to surgery.
IV	Haematogenous metastases or distant lymph node metastases	Haematogenous metastases or distant lymph node metastases
V	Bilateral renal involvement at the time of initial diagnosis. Each side's tumour should be substaged separately according to above criteria	Bilateral renal involvement at the time of initial diagnosis Each side's tumour should be substaged separately according to above criteria.

COG, Children's Oncology Group; SIOP, Société Internationale d'Oncologie Pédiatrique; WT, Wilms' tumour.

a high frequency (25%) of severe chronic and life-threatening health conditions in adult life [8]. The late sequelae may include renal and cardiac dysfunction, hypertension, musculoskeletal abnormalities, metabolic syndrome, impaired fertility, reduced pulmonary function and secondary malignancies [7,109,110]. End-stage renal failure is reported in 1% of unilateral WT and about 10% of patients with bilateral disease at long-term follow-up [111]. The risk of congestive heart failure increases with the cumulative dose of DOX administered, with a critical threshold of 240 mg/m<sup>2</sup>. Cardiotoxicity is potentiated by the concurrent use of radiotherapy, with females and infants more susceptible [109]. Similarly, DOX seems to potentiate the adverse effects related to radiotherapy, likely due to its radiosensitisation of cells. These effects include abnormal tissue growth within the target area and secondary malignancies. In this context, omission of agents likely to cause late effects is important to consider in treatment optimisation. As mentioned previously, the last trial, to demonstrate that a reduction in intensity was acceptable, found DOX does not need to be added in the treatment of stage II–III intermediate-risk WT [5]. Although this reduction marginally increases the risk of

Table 4

Two-year EFS and 5-year overall survival for WT in the SIOP 2001 protocol.

Histopathology	Stage I	Stage II	Stage III	Stage IV	All risk group
<b>Low (N)</b>	95	6	23	61	185
2 y EFS (%)	97	100	100	91	95
5 y OS (%)	99	100	100	94	98
<b>Intermediate (N)</b>	1352	625	514	389	2880
2 y EFS (%)	92	89	88	81	89
5 y OS (%)	98	97	94	89	96
<b>High (N)</b>	163	115	141	75	494
2 y EFS (%)	91	84	68	31	74
5 y OS (%)	97	82	70	35	76
<b>All (N)</b>	1610	746	678	525	3559
2 y EFS (%)	92	88	84	76	87
5 y OS (%)	98	94	90	82	93

EFS, event-free survival; N, number of patients; OS, overall survival; SIOP, Société Internationale d'Oncologie Pédiatrique; WT, Wilms' tumour.

Source: SIOP WT 2001 database for all patients with stage I–IV Wilms' tumour treated as per-protocol with pre-op chemotherapy, 5731 patients were registered from 2001 to 2015. Analysis provided by Harm van Tinteren, trial statistician.



relapse, it likely outweighs the increased risk of severe late effects as the small subset of children that relapse can be rescued. Likewise, omission of lung radiotherapy may also be an acceptable treatment approach for lung lesions that have clearly responded to post-operative chemotherapy [112].

#### 6.4. Targeted therapy and immunotherapy

With our increased understanding of the aberrant molecular pathways that contribute to tumourigenesis comes the drive to identify targeted therapeutics and introduce a more personalised medicine approach.

Preclinical testing has shown that targeting the IGF signalling pathway results in cell cycle arrest, with a consequent reduction in tumour volume in transplanted WT xenografts [113,114]. Despite these encouraging results, a phase II study assessing cixutumumab monotherapy (a monoclonal antibody [mAb] to IGF1R) showed no objective response in refractory or relapsed WT [115]. As this was a single-agent trial, the poor response may be due to the complexity of IGF signalling and its interaction with other oncogenic pathways [116]. As mentioned previously, the WNT/ $\beta$ -catenin pathway is also frequently activated in WT and its downstream effectors play a general role in cancer progression [117]. Although drugs targeting WNT signalling have huge potential, preclinical and clinical studies have not yet been performed. Another promising target is MYCN, as amplification of the oncogene is associated with reduced survival in several childhood cancers including WT [31]. MYCN proteins have been considered undruggable until the recent introduction of a class of inhibitors of Aurora A [30]. These inhibitors destabilise interactions between Aurora A and MYCN and are being tested in several adult phase I and II studies. The sole paediatric phase II study using an Aurora A kinase inhibitor alisertib showed an objective response in only 1 in 10 WT patients [118].

Despite the disappointing results from previous early-phase studies in WT, there are several studies currently recruiting for children with relapsed and refractory solid tumours. Current trials include erlotinib, an epidermal growth factor receptor (EGFR) inhibitor, ramucirumab, a vascular endothelial growth factor receptor (VEGF) inhibitor, talazoparib, a PARP inhibitor and selinexor [119].

The field of immuno-oncology includes a spectrum of interventions that encompass mAbs, cancer vaccines and genetically engineered lymphocytes [120]. Early-phase testing of immunotherapy for refractory and relapsed childhood solid tumours is advancing. One class of therapy to have garnered much attention is antibody–drug conjugates [121]. Lorvotuzumab mertansine, a conjugate between a cytotoxic and an mAb to CD56, showed impressive anti-tumour activity against CD56-positive WT xenografts [122]. CD56 (NCAM-1) is expressed in WT, enriched in blastema, and CD56<sup>+</sup>

ALDH-1<sup>+</sup> cells may act as cancer-initiating stem cells in a subset of tumours [123]. A phase II trial in children has recently commenced [124]. A phase I study of the immune checkpoint inhibitor ipilimumab, an anti-CTLA4 antibody that blocks the inhibitory signal to cytotoxic T cells, has already been undertaken [125]. Finally, *N*-glycosylated gangliosides, including NGcGM3 are an attractive target, due to their expression on several cancers including WT [126]. A phase I trial testing racotumomab, a vaccine targeting NGcGM3, showed immunogenicity and low toxicity, but further trials are needed to assess response as the one patient with anaplastic WT had progressive disease [127]. WT1 is a major target for immunotherapy, given its oncogenic role in several adult cancers, and promising results have been demonstrated with dendritic cell vaccines and gene-modified T-cell therapy [128,129]. Whether these approaches are transferrable to childhood WT remains to be seen. To our knowledge, no trials have been conducted in WT using adoptive engineered T or NK cells.

## 7. Treatment of non-WT

Overall, these tumours and their respective subtypes are very rare and it remains challenging to gather robust evidence for treatment recommendations. Hence, cross-Atlantic collaboration, phase I/II studies and multinational RCTs are extremely important to obtain useful clinical data for these patients.

### 7.1. Clear cell sarcoma of the kidney

As per WT, patients registered on the SIOP-2001 trial were treated with pre-operative chemotherapy. Post-operative treatment consisted of ACT-D/VCR/DOX for patients with local stage I disease and of ifosfamide(IFO)/ETO/carboplatin(CDC)/DOX for patients with stage II–IV disease, with irradiation of the flank for stage II and III patients [130]. The 5-year overall EFS of SIOP 93-01/2001 was 79% and the OS was 86%. Stage IV disease and young age were significant adverse prognostic factors for EFS [130–132].

The planned treatment for CCSK in the upcoming ‘UMBRELLA 2016’ study considers the best available treatment to be ETO/CDC/DOX with alternating CYC/IFO for any tumour stage. Flank radiotherapy will be given to all patients with local stage II and III tumours since the UK-WT-2 study revealed a high local relapse rate in stage II patients treated without radiotherapy [133]. For stage IV patients, if metastases are unresectable, irradiation to metastatic sites will be given; similarly, flank irradiation will be according to local stage.

Although outcomes for CCSK have improved, a subgroup of patients do not fare well, including young patients and those with advanced stage or relapsed disease. As treatment for CCSK is approaching the

maximum tolerated intensity of traditional cytotoxic agents, new targeted therapies are necessary. Possible targets include the Sonic Hedgehog signalling pathway (SMO/GLI1 inhibitors), the PI3K–Akt signalling pathway (PI3K inhibitors, Akt inhibitors, mTOR inhibitors), *EGFR* (erlotinib/gefitinib), *BCOR* (inhibition of *BCOR*) and demethylating agents (e.g. decitabine). Another possibility may be to inhibit the *YWHAE–NUTM2B/E* fusion transcript but targeting fusion transcripts has thus far proven to be difficult.

### 7.2. Malignant rhabdoid tumour of the kidney

Historically, patients with MRTK were treated as high-risk WT, but currently, they are managed according to rhabdoid tumour protocols. In SIOP 93-01/2001 trials, most patients received pre-operative therapy with ACT-D/VCR followed by post-operative chemotherapy, consisting of ETO/CDC/IFO/DOX, and radiotherapy but OS rates remained poor (25–30%) [134]. Similar results have been observed using the North American 1–5 protocols [135]. Younger age was an important adverse prognostic indicator and OS in infants was only 9% [135]. Overall, 30% of patients progressed during initial therapy. A few case reports have demonstrated effectiveness of regimens containing IFO/CDC/ETO alternating with VCR/DOX/CYC in advanced disease and some centres suggest high-dose therapy with stem cell rescue based on these few patients [136–139]. MRTK patients will be treated according to the European Rhabdoid Registry (EU-RHAB) protocol with DOX/IFO/CDC/ETO and VCR/CYC/Dactinomycin. Children under 18 months of age will receive additional CDC/thiotepa and stem cell rescue. Early complete resection is important in the treatment of MRTK.

Novel therapeutic approaches are urgently required to improve outcomes for patients with MRTK. Accumulating evidence suggests that in its native form, the SWI/SNF complex inhibits cell cycle progression by transcriptionally repressing *CCND1* (encodes cyclin D1) and activating p16<sup>INK4A</sup> and p21<sup>CIP</sup> [140–142]. Based on this observation, therapies targeting cyclin D1 and CDK4 have been tested in pre-clinical models of MRTK with some evidence of activity [143]. Loss of *SMARCB1* has also been shown to activate expression of the mitotic regulator Aurora A kinase and the Sonic hedgehog pathway [144,145]. In addition, targets such as *EZH2*, *CXCR4*, *IGF2*, *PD-1/PD-L1* and the *INI1* pathway should be considered for future treatment strategies [146].

### 7.3. Renal cell carcinoma

Irrespective of subtype, the mainstay of therapy for paediatric RCC is resection. Many patients with localised, completely resected disease are cured without adjuvant therapy. In translocation RCC, morphology (Fuhrman grade) and distant metastatic disease were

associated with poor survival [147]. In children, local lymph node involvement seems not to be associated with unfavourable outcome, even without adjuvant therapy [148]. The need for radical node dissection still remains to be determined [149]. Patient survival outcomes reported in paediatric RCC patients are above 90% for stage I and II, around 60% for stage III and around 30% for stage IV [150,151].

Although there is no standard treatment for unresectable metastatic RCC in children, the approach is in alignment with recently published adult RCC guidelines [152]. High-dose interleukin-2 has had some success but response is only observed for clear cell RCC [147,153]. Recent data on translocation RCC suggest that VEGF-targeted therapy may be the best therapeutic option, with sunitinib probably being the most effective [154,155]. Another relevant target is the mTOR pathway as high levels of phosphorylated S6, a marker for mTOR activity are found in RCC [73]. Patients who had progressive disease on VEGF therapy and were switched to mTOR inhibitors (everolimus, temsirolimus) showed at least transient disease stabilisation, including one with a partial response. The fusion protein *ASPL–TFE3* transactivates the promoter of the *MET* receptor tyrosine kinase (RTK), therefore inhibiting this RTK may be a potential therapy for this type of translocation RCC [156]. Indications for chemotherapy are extremely rare, but responses to gemcitabine/DOX/oxaliplatin have been observed [148].

## 8. Adult WT

WT is rare in patients over 16 years of age, with only 70 adults diagnosed in Europe each year [157]. The clinical presentation in older patients is commonly flank pain and weight loss. There are no specific adult treatment protocols but cure has been achieved using paediatric regimens [158,159]. Adults have higher treatment-related toxicity, with VCR neuropathy as the main adverse event. Five-year survival is stage dependent; the largest study showed variation from 74% for stage I disease to less than 15% for metastatic tumours [157]. The poorer prognosis may, in part, be explained by diagnostic delay. Many adult WT patients are initially misdiagnosed as there are no specific radiological findings to differentiate WT from RCC. Central pathology review is recommended as histological diagnosis can be challenging, particularly for pathologists unfamiliar with paediatric tumours. Best practice guidance, based on international consensus, has been published to hasten time to start adjuvant therapy and improve outcome [160].

## 9. Challenging renal tumours—a patient with WT

For challenging patients, national and international multidisciplinary expert panels are essential to advise on optimum treatment and to minimise inequalities

in childhood cancer survival across Europe. Through the upcoming European expert paediatric oncology reference network projects, centres with expertise in a particular treatment modality could be identified to provide cross-border treatment of complex renal and other tumours [161].

The most challenging renal tumours are predominantly the non-WT and WT that are high-risk histology, bilateral, relapsed or refractory, metastatic and/or with thrombus extension into the inferior vena cava. Each of these scenarios occurs in about one in ten cases and some patients may present with several concomitant complex features. In the following clinical example, we try to highlight some of the key decisions to consider during treatment of complicated WT.

*Example: A child with a clinical history of extended abdomen, weight loss and haematuria. CT describes a large solid/necrotic mass (volume ~1100 ml) in the left kidney with thrombus extension into the inferior vena cava at suprahepatic level and three minor lesions in the right kidney. No signs of rupture or abnormal lymph nodes or spread to other organs. Chest considered clear but with a single polygonal minor lesion (3 mm) in lower right pulmonary lobe. Renal function is within the normal range.*

Key issues at this stage would be 1) whether to treat pre-operatively as metastatic due to the lung lesion and add DOX to VCR/ACT-D, 2) to discuss surgical approach with an experienced team due to thrombus and bilateral disease, and 3) to consider diffusion weighted MRI in order to better characterise the right-sided renal lesions and the thrombus.

There remains great uncertainty surrounding the interpretation and treatment of minor lung lesions (<5 mm) detected on CT, despite many attempts to predict the likelihood of metastatic disease, based on size, number, appearance and location of nodules [162]. Accordingly, the decision to add DOX pre-operatively is debatable. Owing to this grey area, it is recommended to biopsy small lung lesions of uncertain significance if feasible.

In cases with bilateral disease, the goal is to preserve renal function without compromising the risk of relapse. An experienced surgical team should discuss NSS versus nephrectomy or biopsy of the right kidney for histology to assess if the lesions only contain nephrogenic rests, potentially negating the need for further surgery. For patients with inferior vena cava thrombus extension, in whom thrombectomy is considered, bypass support from cardiothoracic surgeons is essential. An additional high-quality diffusion-weighted MRI would be optimal to reliably assess the extent of disease and to plan surgery.

*After chemotherapy, pre-operative MRI illustrated that the tumour size was unchanged and that liver lesions had appeared. CT showed that the lung lesion was slightly bigger (6 mm).*

The key issue now is progressive disease. A decision needs to be made whether to operate now, considering the risks of challenging nephrectomy and thrombectomy,

or whether to change and intensify chemotherapy with the aim of reducing these risks. Pulmonary metastectomy in the same surgical séance should also be considered.

*Left nephrectomy including thrombectomy at week 7 was performed. Histopathology showed 25% necrosis and viable tumour with mainly blastemal components (high-risk) and lymph node with viable tumour (stage III). The patient was started on the high-risk treatment protocol and received local radiotherapy. Lung and liver lesions were carefully monitored.*

This patient represents a group with poor prognosis due to chemo-resistant progressive blastemal disease. Intensified chemotherapy, including DOX, and radiotherapy are essential. The choice of intensified chemotherapy, or even high-dose chemotherapy with stem cell rescue, remains in debate due to insufficient data from case series. In future, this group of patients may well benefit from a personalised medicine approach with sequencing of their tumours undertaken to identify actionable mutations and direct targeted therapy.

## 10. Discussion

The treatment of WT is one of the successes of paediatric oncology, with both the SIOP and COG approaches achieving cure in approximately 90% of patients. The focus has now shifted to reducing therapeutic burden and the late effects related to anthracyclines and radiotherapy. A recent analysis of late mortality in childhood cancer survivors showed that we have already made progress, with a reduction in secondary neoplasms and cardiac events corresponding to a reduction in treatment intensity [163]. Better risk stratification is required to identify which subgroups of patients can benefit from further treatment reduction.

In contrast, approximately 10% of children do not survive WT, corresponding to at least 100 annual deaths in Europe but with OS varying between European countries. Continued preclinical research is needed to identify new agents for relapsed and refractory WT, prior to testing their efficacy in early phase trials. Targeted therapy has, so far, had less promising results in clinical studies but the studies were limited to small numbers of patients with tumours that had not undergone genetic characterisation. Owing to the complex interactions between signalling pathways and resistance mechanisms, rational combination therapies are needed. Treatment strategies will be influenced by our improved understanding of Wilms' tumorigenesis, gained through upcoming international studies and the increasing numbers of tumours that will undoubtedly be sequenced over the next few years.

Genetic analysis of tumours and correlation with clinicopathological characteristics can identify both prognostic factors and potentially druggable targets.

However, the efficacy of any intervention should still be assessed through RCTs in order to gain robust evidence for clinical practice. In some subgroups, the feasibility of large trials is hampered by patient numbers, which further stresses the need for expanding the number of countries participating in SIOP and COG protocols and for continued cross-Atlantic collaboration. It is important that steering committees within each group collaborate, prioritise and support the most relevant research questions to be addressed. Treatment outcomes in non-WT renal cancer have lagged behind and in rhabdoid tumour, in particular, the prognosis remains dismal. The numbers of children with these high-risk tumours are insufficient to allow large RCTs, and to circumvent this, COG and SIOP are collaborating on a number of upcoming small studies.

Overall, more than half of all relapses in WT occur in children in the intermediate-risk group, which indicates that our current stratification methods need refinement. Work is ongoing to identify tumour and circulating biomarkers that have clinicopathological associations and to validate these markers in international studies. Follow-up for patients is currently limited to clinical assessment and imaging for 2–5 years depending on stage and histology. The question remains whether liquid biopsy has a role to play in non-invasive monitoring and whether in future, it may be used routinely to identify disease reoccurrence prior to clinical relapse [164].

For 9 in 10 children diagnosed with a WT in Europe, it is now a curable cancer. Continued effort is needed to improve the poorer outcomes in high-risk metastatic WT, relapsed WT and the high-risk non-Wilms' renal cancers. For this to be achieved requires the international community to work together, both in the laboratory and in paediatric oncology units.

#### Conflict of interest statement

None declared.

#### Acknowledgements

The authors thank the SIOP-RTSG, particularly Harm van Tinteren, trial statistician, for permission to present unpublished data from the group's overall registration database.

TT and KPJ are funded by Great Ormond Street Hospital Biomedical Research Council and TT receives additional funding from The Crick. JB is funded by Great Ormond Street Hospital Children's Charity (512899) and the European Expert Paediatric Oncology Reference Network for Diagnostics and Treatment (508475). SLMG was funded by the Pediatric Oncology Support Rotterdam Foundation (sKOCR) and DaDa Foundation.

#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejca.2016.09.005>.

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