Table 1
Biopsy type by national centre

Group	**No biopsy**		**FNA**		**PCNB**		**Open biopsy**	
	No.	***%***	No.	***%***	No.	***%***	No.	***%***
Total	2,002	***67***	288	***10***	620	***21***	61	***2***
Brazil	257	***79***	29	***9***	30	***8***	8	***3***
Germany	694	***92***	60	***8***	0	***0***	0	***0***
France	477	***79***	78	***13***	39	***6***	12	***2***
SIOP-NL	558	***74***	87	***12***	92	***12***	14	***2***
UK	16	***3***	34	***6***	459	****86***	27	***5***

* UK continued its established national practice from the UKW3 trial of recommending percutaneous cutting needle biopsy (PCNB) prior to chemotherapy in all patients. FNA: fine needle aspirate.

Table 2
Patient and tumour characteristics by biopsy group.

Biopsy	No	Yes	P values
Total	2002	969	
Sex			0.7
Male	933 (46.7%)	444 (45.9%)	
Female	1065 (53.3%)	524 (54.1%)	
Age			**< 0.001**
0.5-1.99	567 (28.3%)	192 (19.8%)	
2-3.99	718 (35.9%)	317 (32.7%)	
4-17.5	717 (35.8%)	460 (47.5%)	
Category			**< 0.001**
Localized	1752 (87.5%)	777 (80.2%)	
Metastatic	250 (12.5%)	192 (19.8%)	
Side			0.187
Right	953 (47.6%)	487 (50.3%)	
Left	1049 (52.4%)	482 (49.7%)	
Volume at diagnosis (ml) (Median & IQR)	410 (220-670)	570 (310-880)	**< 0.001**
Volume at surgery (ml) (Median & IQR)	140 (50-300)	180 (70-410)	**< 0.001**
Abdominal stage			**< 0.001**
I	1025 (53.6%)	374 (40.0%)	
II	455 (23.8%)	227 (24.3%)	
III	433 (22.6%)	334 (35.7%)	
Margin status			**<0.001**
Negative	1782 (89.0%)	766 (79.1%)	
Positive	220 (11.0%)	203 (20.9%)	
Lymph node status			**<0.001**
Negative	1793 (89.6%)	817 (84.3%)	
Positive	209 (10.4%)	152 (15.7%)	
Tumour rupture			**0.004**
No	1339 (91.1%)	739 (87.2%)	
Yes	131 (8.9%)	108 (12.8%)	
Histological Risk group (panel review/local pathol.)			0.027
Low Risk	111 (5.8%)	54 (5.9%)	
Intermediate Risk	1558 (81.9%)	731 (78.3%)	
High Risk	234 (12.3%)	149 (15.8%)	

Table 3
Relapses and deaths by biopsy group

	No Biopsy (n = 2002)	**Biopsy (n = 969)**	**Total (n = 2971)**
Local relapse only	45 (2.2%)	41 (4.2%)	86 (2.9%)
Combined relapse	31 (1.5%)	22 (2.3%)	53 (1.8%)
All Local relapses	**76 (3.8%)**	**63 (6.5%)**	**139 (4.7%)**
Distant relapse only	131 (6.5%)	91 (9.4%)	222 (7.5%)
Unknown site	49 (2.4%)	10 (1.0%)	59 (2.0%)
Any relapse	**256 (12.8%)**	**164 (16.9%)**	**420 (14.1%)**
Death	107 (5.3%)	97 (10.0%)	204 (6.9%)

Table 4
Univariate and multivariable analysis of risk factors of recurrence for any local relapse.

		No. with local relapse	Univariate analysis			Multivariable analysis		
			HR	95% CI	*p*-value	HR	95% CI	*p*-value
Biopsy	No	76	1		0.002	1		0.13
	Yes	63	1.70	1.21-2.37		1.40	0.9-2.17	
Age	0.5 – 1.99	14	1		<0.001	1		
(years)	2 – 3.99	46	2.37	1.30-4.32		2.24	1.22-4.09	0.01
	4 – 17.5	79	3.60	2.04-6.36		2.78	1.55-4.99	0.001
Risk	Low + Intermediate	100	1		<0.0001	1		
	High	39	2.83	1.95-4.1		2.32	1.58-3.42	<0.0001
Volume at surgery	(/100ml)	139	1.11	1.07-1.16	<0.0001	1.07	1.02-1.12	0.01
Volume at diagnosis	(/100ml)	139	1.09	1.06-1.13	<0.0001			
Lymph node	Negative	114	1		0.03			
status	Positive	25	1.60	1.03-2.46				
Tumour	No	119	1		0.004			
Rupture*	Yes	20	1.96	1.22-3.15				
Abdominal	I	59	1		0.06			
stage	II	32	1.14	0.74-1.76				
	III	48	1.57	1.07-2.30				
Site	Right	67	1		0.94			
	Left	72	1.01	0.72-1.41				
Margin	Negative	117	1		0.62			
status	Positive	22	1.12	0.71-1.76				
Gender	Male	61	1		0.57			
	Female	78	1.10	0.78-1.54				
Category	Localized	114	1		0.25			
	Metastatic	25	1.28	0.83-1.98				
Biopsy done	no	76	1		0.0002			
	Fine needle	17	1.61	0.95-2.74				
	Trucut	37	1.52	1.03-2.26				
	Open	9	3.92	1.96-7.83				

* in a sensitivity analysis in which the value of ‘yes’ rather than ‘no’ was imputed for the patients with missing data for ‘rupture at surgery’, the hazard ratio and p value for any

association of local recurrence with biopsy were 1.175 and 0.37, respectively, adding further weight to the lack of significant association of this parameter in multi-variable analysis.

Table 5
Univariate and multivariable analysis of risk factors for local recurrence restricted to patients with intermediate risk stage I and II WT that received no doxorubicin and no radiotherapy (n=797)*

		No. with local relapse	**Univariate analysis**			**Multivariable analysis**		
			HR	95% CI	*p*-value	HR	95% CI	*p*-value
Biopsy	No	13	1		0.01	1		0.06
	Yes	17	2.65	1.28-5.45		2.40	0.95-6.05	
Age	0.5 – 1.99	6	1		0.12	1		
(years)	2 – 3.99	11	1.81	0.67-4.90		1.80	0.67-4.88	0.25
	4 – 17.5	13	2.68	1.02-7.05		2.31	0.87-6.14	0.09
Biopsy done	no	13	1		0.05			
	Fine needle	4	2.29	0.74-7.03				
	Trucut	13	2.78	1.29-6.00				
Volume at surgery	(/100ml)	30	1.004	0.88-1.14	0.96			
Volume at diagnosis	(/100ml)	30	1.06	0.97-1.17	0.16			
Abdominal	I	22	1		0.29			
stage	II	8	1.54	0.68-3.74				
Site	Right	14	1		0.82			
	Left	16	1.08	0.53-2.23				
Gender	Male	12	1		0.32			
	Female	18	1.44	0.69-3.00				

* This cohort excluded any patients documented to have had open biopsy, positive lymph nodes, tumour capsule rupture and those who received radiotherapy to the flank or ‘high-risk’ post-operative chemotherapy regimens.

Supplemental Table 1
Pre-operative and post-operative chemotherapy and radiotherapy protocol

Biopsy		**No (n = 2002)**	**Yes (n = 969)**
Pre-operative chemotherapy*			
	AV	1719 (86%)	727 (75%)
	AVD	281 (14%)	242 (25%)
	Other	2 (0%)	0
Post-operative chemotherapy*			
	AV/AV1	724 (38%)	284 (32%)
	AV2	249 (13%)	102 (12%)
	AVD	650 (35%)	302 (34%)
	CCED	219 (12%)	180 (20%)
	None	34 (2%)	18 (2%)
Radiotherapy			
	Yes	446 (22%)	348 (36%)
	No	1556 (78%)	621 (64%)
	Flank radiotherapy	329 (16.4%)	302 (31.2%)
	Lung radiotherapy	51 (2.5%)	85 (8.8%)

* Drug regimen abbreviations: AV/AV-1 is actinomycin-D and Vincristine given over 4 weeks; AV-2 is actinomycin-D and vincristine given over a 27 week period; AVD is actinomycin-D, vincristine and doxorubicin given over 6 weeks (pre-op) and 27 weeks (post-op); CCED is alternating courses of cyclophosphamide/doxorubicin with carboplatin/etoposide given over a 35 week period.

Supplemental Table 2
Univariate and multivariable analysis of risk factors of recurrence for any distant relapse.

		Events	Univariate analysis			Multivariable analysis		
			HR	95% CI	*p*-value	HR	95% CI	*p*-value
Biopsy	No	162	1		0.0026	1		0.97
	Yes	113	1.44	1.13-1.84		0.99	0.72-1.37	
Age	0.5 – 1.99	26	1		<0.0001	1		
(years)	2 – 3.99	86	2.41	1.55-3.74		2.01	1.29-3.14	0.002
	4 – 17.5	163	4.11	2.71-6.22		2.63	1.71-4.04	<0.0001
Histological risk group	Low + Intermediate	190	1		<0.0001	1		<0.0001
	High	85	3.38	2.62-4.37		2.82	2.15-3.7	
Abdominal	I	84	1		<0.0001	1		
stage	II	74	1.89	1.38-2.58		1.58	1.15-2.17	0.005
	III	117	2.78	2.10-3.68		1.52	1-2.32	0.05
Category	Localized	186	1		<0.0001	1		<0.0001
	Metastatic	89	2.98	2.32-3.84		2.5	1.88-3.31	
Volume at surgery	(per 100ml)	275	1.10	1.07-1.13	<0.0001	1.08	1.04-1.12	<0.0001
Type of	No	162	1		0.02			
biopsy	Fine needle	31	1.39	0.94-2.04				
	Cutting needle	73	1.43	1.08-1.88				
	Open	9	1.84	0.94-3.60				
Volume at diagnosis	(per 100ml)	275	1.08	1.06-1.11	<0.0001			
Lymph node	Negative	204	1		<0.0001			
status	Positive	71	2.63	2.00-3.44				
Site	Right	113	1		0.01			
	Left	162	1.35	1.06-1.72				
Margin	Negative	215	1		0.0002			
status	Positive	60	1.70	1.27-2.26				
Tumour	No	254	1		0.8			
rupture	Yes	21	0.94	0.60-1.47				
Gender	Male	127	1		0.99			

	Female	148	1.00	0.79-1.26

Supplemental Table 3
5-year survival with or without inclusion of fine needle aspirates in the biopsy group (univariate analysis)

Biopsy		**No**	**Yes**	**P values**
		%, CI 95%	**%, CI 95%**	
5y local relapse free survival	FNA in	95.9 94.9 − 96.8	93.1 91.5 − 94.8	0.002
	FNA out	95.5 94.6 − 96.4	93.1 91.1 − 95.1	0.01
5y distant relapse free survival	FNA in	91.3 90.0 − 92.6	87.6 85.5 − 89.8	0.002
	FNA out	90.8 89.5 − 92.1	87.7 85.2 − 90.3	0.01
5y EFS	FNA in	86.5 85.0 − 88.1	81.0 78.5 − 83.6	0.001
	FNA out	86.0 84.5-87.5	80.5 77.6-83.6	0.001
5y OS	FNA in	94.2 93.1 − 95.3	89.3 87.3 − 91.3	<0.0001
	FNA out	93.8 92.8 − 94.9	88.5 86.1 − 91.0	<0.0001

FNA in (n=2002 in the no biopsy group, n=969 in the biopsy group), FNA out (n=2290 in the no biopsy group, n=681 in the biopsy group). In both scenarios, the 'biopsy – yes' group includes both percutaneous cutting needle biopsy and open biopsy.

Supplemental Table 4
Univariate and multivariable analysis of risk factors of recurrence for overall survival.

		Events	Univariate analysis			Multivariable analysis		
			HR	95% CI	*p*-value	HR	95% CI	*p*-value
Biopsy	No	107	1		<0.0001	1		0.51
	Yes	97	1.88	1.43-2.48		1.13	0.79-1.62	
Age	0.5 – 1.99	24	1		<0.0001	1		
(years)	2 – 3.99	55	1.65	1.02-2.67		1.17	0.72-1.9	0.54
	4 – 17.5	125	3.35	2.17-5.2		1.64	1.03-2.58	0.04
Histological risk group	Low + Intermediate	117	1		<0.0001	1		<0.0001
	High	87	5.62	4.26-7.42		4.82	3.58-6.47	
Abdominal	I	52	1		<0.0001	1		
stage	II	41	1.70	1.13-2.56		1.38	0.91-2.1	0.13
	III	111	4.33	3.11-6.02		2.32	1.45-3.7	0.0004
Category	Localized	125	1		<0.0001	1		<0.0001
	Metastatic	79	3.86	2.91-5.12		3.4	2.48-4.66	
Volume at surgery	(per 100ml)	204	1.12	1.09-1.16	<0.0001	1.08	1.04-1.13	<0.0001
Tumour	No	167	1		<0.0001	1		0.03
rupture	Yes	37	2.62	1.83-3.74		1.52	1.04-2.22	
Type of	No	107	1		<0.0001			
biopsy	Fine needle	22	1.49	0.94-2.36				
	Cutting needle	64	1.91	1.40-2.60				
	Open	11	3.50	1.88-6.50				
Volume at diagnosis	(per 100ml)	204	1.10	1.07-1.13	<0.0001			
Lymph node	Negative	141	1		<0.0001			
status	Positive	63	3.42	2.54-4.61				
Site	Right	91	1		0.26			
	Left	113	1.17	0.88-1.54				
Margin	Negative	149	1		<0.0001			
status	Positive	55	2.27	1.66-3.09				
Gender	Male	85	1		0.15			

	Female	119	1.22	0.92-1.61				

Supplemental Table 5
Univariate and multivariable analysis of risk factors of recurrence for event free survival.

		Events	Univariate analysis			Multivariable analysis		
			HR	95% CI	*p*-value	HR	95% CI	*p*-value
Biopsy	No	275	1		0.0006	1		0.46
	Yes	180	1.39	1.15-1.67		1.1	0.85-1.42	
Age	0.5 – 1.99	66	1		<0.0001	1		
(years)	2 – 3.99	142	1.60	1.19-2.14		1.43	1.06-1.92	0.02
	4 – 17.5	247	2.52	1.92-3.31		1.84	1.38-2.45	<0.0001
Histological risk group	Low + Inetrmediate	340	1		<0.0001	1		<0.0001
	High	115	2.59	2.1-3.20		2.27	1.82-2.83	
Category	Localized	337	1		<0.0001	1		<0.0001
	Metastatic	118	2.18	1.77-2.7		1.91	1.51-2.41	
Volume at surgery	(per 100ml)	455	1.10	1.07-1.12	<0.0001	1.08	1.05-1.11	<0.0001
Type of biopsy	No	275	1		0.0003			
	Fine needle	45	1.21	0.88-1.66				
	Cutting needle	117	1.38	1.11-1.71				
	Open	18	2.31	1.43-3.73				
Abdominal stage	I	176	1		<0.0001			
	II	105	1.30	1.02-1.65				
	III	174	2.00	1.62-2.47				
Volume at diagnosis	(per 100ml)	455	1.08	1.05-1.10	<0.0001			
Lymph node status	Negative	359	1		<0.0001			
	Positive	96	2.07	1.65-2.59				
Margin status	Negative	366	1		0.001			
	Positive	89	1.44	1.07-1.93				
Tumour rupture	No	405	1		0.01			
	Yes	50	1.44	1.07-1.93				
Site	Right	206	1		0.13			
	Left	249	1.15	0.95-1.38				
Gender	Male	213	1		0.76			

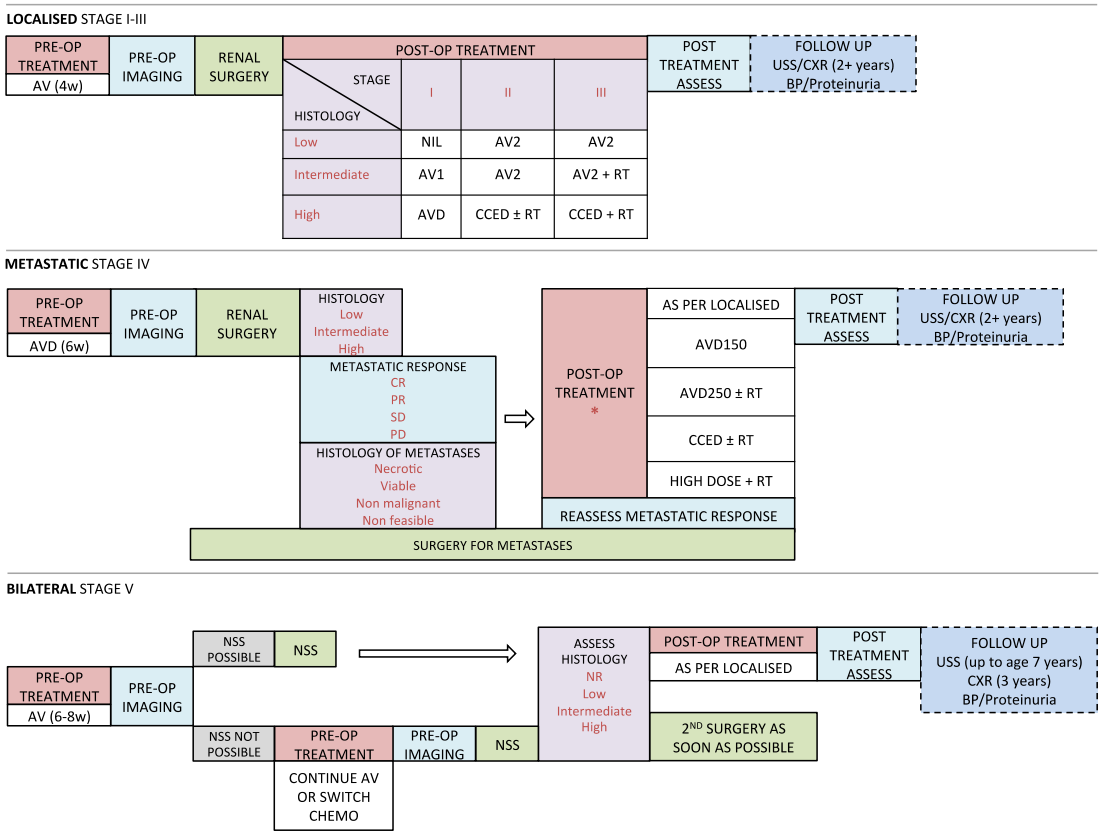
	Female	242	0.97	0.80-1.16

Supplemental Table 6
Hazard ratio for biopsy between the whole population and a subgroup excluding the open biopsy cases.

Outcome	**Statistical analysis**	**Risk for biopsy**					
		Whole population n=2971			**Population with no open biopsy n=2910**		
		HR	95%CI	p-value	HR	95%CI	p-value
Local relapse	Univariate	1.70	1.21-2.37	0.002	1.55	1.09-2.20	0.01
	Multivariable	1.40	0.9-2.17	0.13	1.45	0.92-2.27	0.11
Distant relapse	Univariate	1.44	1.13-1.84	0.0026	1.42	1.11-1.81	0.005
	Multivariable	0.99	0.72-1.37	0.97	1.00	0.72-1.39	0.99
EFS	Univariate	1.39	1.15-1.67	0.0006	1.33	1.09-1.61	0.004
	Multivariable	1.1	0.85-1.42	0.46	1.08	0.83-1.41	0.55
OS	Univariate	1.88	1.43-2.48	<0.0001	1.78	1.34-2.36	<0.001
	Multivariable	1.13	0.79-1.62	0.51	1.13	0.78-1.65	0.51

Supplementary Figure 1

Treatment flow-chart for localised, metastatic and bilateral WT as per SIOP-RTSG protocol.



AV (ACT-D/VCR 4 weeks), AV1 (ACT-D/VCR 4 weeks), AV2 (ACT-D/VCR 27 weeks), AVD (ACT-D/VCR/DOX 27 weeks), AVD150 (ACT-D/VCR/DOX 27 weeks, cumulative DOX including pre-op treatment 150mg/m^2), AVD250 (ACT-D/VCR/DOX 27 weeks, cumulative DOX including pre-op treatment 250mg/m^2), CCED (CYC/CDC/ETO/DOX 35 weeks), RT (radiotherapy), NSS (nephron sparing surgery), NR (nephrogenic rest), CXR (chest X-ray), USS (ultrasound), BP (blood pressure), CR (complete response), PR (partial response), SD (stable disease), PD (progressive disease) from RECIST guideline.

** The post-operative treatment of metastatic WT is complex and depends on multiple factors including histopathology, location of metastases, overall response of metastases and the timing and feasibility of surgery for metastases.*

Reproduced from Brok J, Treger T, Gooskens S, van den Heuvel-Eibrink M, Pritchard-Jones K Biology and treatment of renal tumours of childhood. European J Cancer (2016) 68:179-195 (supplementary data, open access) DOI: https://doi.org/10.1016/j.ejca.2016.09.005

Highlights

- Biopsy is not associated with local relapse in multivariable analysis.
- Results for local relapse are similar for fine needle or cutting needle biopsy.
- Automatic ‘upstaging’ is unnecessary in needle biopsied Wilms tumour.
- Open biopsy may carry greater risk and should not be used routinely.
- Biopsy should be reserved for cases with atypical clinical or radiologic features.