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The Clinical Impact of Somatic Copy Number Variations in Patients With Stage IV Wilms Tumor Enrolled in the SIOP 2001 Trial and Study

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ABSTRACT

Background: Recent research elucidated the prognostic significance of molecular biology in Wilms tumor (WT) by linking somatic genomic variants (such as gain of chromosome 1q) to unfavorable patient outcomes. This analysis describes the clinical impact of copy number variations (CNV) in tumor samples of WT patients with stage IV disease.

Methods: Tumor samples of 55 WT patients with stage IV disease from the United Kingdom, France, and Germany enrolled in the SIOP 2001 study and treated with preoperative chemotherapy (pCHT) were examined for their CNVs of chromosome 1q and other regions of interest using multiplex ligation-dependent probe amplification (MLPA). The identified CNV were analyzed regarding their prognostic impact.

Results: Chromosome 1q gain (1q+) and *TP53* loss occurred in 38.2% and 16.4% of tumors and were associated with older patient age at diagnosis (median [months]: 65 and 64 vs. 49 each, $p = 0.03$ and 0.02, respectively) and poorer 5-year event-free survival (40.0% and 11.1% vs. 67.7% and 82.6%, $p = 0.04$ and <0.01, respectively) compared to their specific control group of tumors without the respective CNV. In patients with pulmonary-only metastasis, 1q+ was an adverse prognostic marker irrespective of remission status after pCHT with or without metastasectomy. A simultaneous *MYCN* gain occurred more frequently in tumors with 1q+ than in tumors without 1q+ ($p = 0.03$). *TP53* loss was linked to high-risk histology and inferior 5-year overall survival ($p < 0.001$).

Abbreviations: CNV, copy number variations; COG, Children's Oncology Group; CR, complete remission; CT, computed tomography; DA, diffuse anaplasia; EFS, event-free survival; HR, high risk; IR, intermediate risk; LOH, loss of heterozygosity; OS, overall survival; pCHT, preoperative chemotherapy; pRT, pulmonary RT; RT, radiation therapy; SIOP-RTSG, International Society of Pediatric Oncology Renal Tumor Study Group; TV, tumor volume; WT, Wilms tumor.

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Conclusions: We confirm the prognostic relevance of 1q+ and *TP53* loss in stage IV WTs and emphasize their potential utility for future treatment stratification.

1 | Introduction

Wilms tumor (WT) is the most common kidney tumor in children younger than 15 years with a prevalence of over 85% [1, 2]. At least 12% of WT patients in Europe present with distant metastasis [3–7]. The most common metastatic site is the lung (85%–95%), followed by the liver (approximately 15%) and other sites (<5%) including abdominal, bone, and brain metastasis [3, 4, 8]. Bone metastases are present in only 0.4% of WT patients and are associated with poor prognosis [9].

The overall survival (OS) for WT of all stages is approximately 90% for newly diagnosed cases [7, 10, 11] and 15%–79% for patients who relapse, with those with high-risk (HR) histology at relapse doing significantly worse [12–16]. The prognosis of metastatic patients treated with preoperative chemotherapy (pCHT) is less favorable with a 5-year OS of 82%–83% compared to patients with localized WT (5-year OS of 90%–98% depending on local stage), although stage IV patients receive more intensive treatment compared to patients with localized disease [3, 17, 18].

WT is treated according to either the SIOP-RTSG (International Society of Pediatric Oncology Renal Tumor Study Group) or the North American Children's Oncology Group (COG) protocols. The SIOP-RTSG has been following the approach of administering 4 weeks of pCHT as standard of care for localized renal tumors since the completion of the SIOP-9 study in 1991 [5, 19]. Metastatic patients initially receive 6 weeks of pCHT, followed by surgery. Postoperative treatment is based on histology, local abdominal stage, and postoperative remission status of metastases [5, 11, 13, 20].

In the SIOP-RTSG protocols, age, histological subtypes, tumor stage, preoperative tumor volume (TV), and for stage IV patients, the metastatic remission status after pCHT are among the relevant prognostic factors for treatment stratification of WT [3, 11, 17, 21–26]. Molecular markers, including copy number variations (CNV), have not yet been implemented for treatment stratification in the SIOP-RTSG protocols [13]. Chagtai et al. showed that 28% of 586 WT (stage I–IV) patients treated with pCHT harbored 1q+, and these were associated with an approximately two-fold higher risk of relapse [27]. In addition, further genomic CNVs with adverse prognostic impact have been identified in WT, notably *TP53* loss at 17p, *MYCN* gain and loss of 1p, and loss of 16q [27–32]. Besides CNVs, driver mutations in several other genes involved in WT tumorigenesis such as *WT1, CTNNB1, TP53, MYCN, SIX1/2, DROSHA*, and *DGCR8* are potentially associated with outcome [33–36].

In this study, we investigated in detail the prognostic significance of genomic CNVs in patients with stage IV WT, as a subgroup of the patients described by Chagtai et al. [27].

2 | Materials and Methods

2.1 | Patients and Cohorts

Patients were eligible for study, if (i) they were registered in the SIOP 2001 study after informed consent; (ii) enrolled in the UK, France, or Germany; (iii) diagnosed with stage IV WT; and (iv) had molecular analysis by multiplex ligation-dependent probe amplification (MLPA) of a fresh-frozen tumor sample as performed and reported by Chagtai et al. [27]. Metastatic disease was defined according to SIOP stage IV criteria [5]. Pulmonary metastasis had to be detectable on the chest x-ray, and patients with computed tomography (CT)-only nodules as defined in Smets et al. were not eligible for the analysis, as these constitute a separate subgroup with a different outcome compared to patients with overt metastasis [37, 38].

2.2 | Diagnostic and Therapeutic Guidelines of the SIOP 2001 Study

Once a WT was suspected, patients routinely underwent abdominal cross-sectional imaging (MRI or CT) and conventional chest x-ray ± chest CT for pulmonary staging to determine the extent of disease. Initial and preoperative TVs were assessed by imaging (MRI, CT, or ultrasound) and calculated using the ellipsoid formula (TV = Length × Height × Depth × 0.523).

The patients were treated according to the international SIOP treatment guidelines with vincristine (V), actinomycin (A), and doxorubicin (D) for 6 weeks, followed by surgery as per guidelines [5, 6]. After pCHT and tumor-nephrectomy with lymph node sampling, the histologic subtype and local staging were determined according to the SIOP-RTSG histology classification for renal tumors [39]. Moreover, the remission status of metastases was assessed radiologically by CT with two outcomes: metastases (i) absent or completely excised (complete remission [CR]); or (ii) incompletely excised or inoperable (non-CR). Depending on these parameters, the patients of group (i) continued with AVD as standard postoperative treatment, while group (ii) and patients with HR histology received intensified four-drug regimen with VP-16 (V), carboplatin (C), cyclophosphamide (C), and doxorubicin (D). Patients in non-CR usually underwent pulmonary biopsy. If no viable tumor cells in a representative number of metastasis were identified, no treatment intensification was applied [6].

Patients with inoperable metastasis at Week 6 after tumor nephrectomy and/or HR histology received radiotherapy (RT) to the metastatic site. In case of pulmonary metastasis, whole lung RT has been recommended in the protocol [3, 5], whilst all patients with local stage III intermediate-risk (IR) or HR

tumors and local stage II diffuse anaplasia (DA) received flank RT.

2.3 | Statistics and Data Analysis of the Stage IV Cohort

The data were extracted from the SIOP 2001 database, completed by queries to the local sites for missing clinical data and imported in IBM SPSS statistics version 29. Statistical analyses were performed on the clinical and tumor-related variables of interest. Subsequently, comparisons were performed using Pearson's chi-square test or Fisher's exact test depending on expected values of greater than 5 or ≤5 for categorical variables, respectively, and Wilcoxon rank-sum test for non-normally distributed variables to determine significant associations between the respective CNVs and between CNVs and the clinical parameters. We classified the tumors into those with the respective CNV of interest (1q+, *TP53* loss, *MYCN* gain) and those in which the respective CNV was absent (other status). For survival and time-to-event analysis, Kaplan–Meier lifetables were generated, and differences between survival curves were tested using log rank test. Two-sided *p*-values below 0.05 were considered statistically significant.

3 | Results

3.1 | Patient and Tumor Characteristics

In the SIOP 2001 study, 373 patients with stage IV WT from the United Kingdom (110), France (118), and Germany (145) were enrolled, out of which 65 patients had fresh frozen samples available for this study. Ten patients with CT-only nodules and/or incomplete data were excluded resulting in 55 patients, including 15 patients from the United Kingdom, 22 from France, and 18 from Germany. The median age of these patients was 54 months (interquartile range [IQR]: 35–65 months) and 50.9% were males (Table 1). Ninety-two percent of patients received the preoperative standard-of-care AVD treatment for 6 weeks, and 5.6% received only 4 weeks of AV (physician's choice) (Table 1). A median TV reduction of 78% (range: 57–86) was observed after pCHT, from a median initial TV of 720 mL (IQR: 504–985 mL) to a median TV at surgery of 175 mL (IQR: 76–348). IR and HR histology represented 80.0% and 20.0%, respectively. All patients with HR histology had DA. The local stage distribution was 25.5%, 20.0%, and 54.5% for abdominal stage I, II, and III, respectively. Postoperative CHT consisted of 55.8% AVD and 36.5% for the VCCD HR regimen. Only 7.7% of patients had a different kind of treatment (physician's choice) (Table 1).

Data on RT were available in 50 patients, of whom 33 (66.0%) underwent RT. Fourteen (28.0%) received local RT, either to the flank or to the whole abdomen. Nineteen (38.0%) patients were irradiated to the metastatic site, seven of them received only pulmonary RT (pRT), nine pRT in combination with local RT to the flank or abdomen, and three patients received pRT as well as RT to one other site with or without liver RT. Seventeen patients (34.0%) were treated with CHT and surgery only, without RT.

Three of the 22 patients with metastatic CR and one of 14 patients with non-CR and available data on RT received pRT.

Twenty-two patients out of 55 had an event, and 40 patients were still alive after an average follow-up period of 5 years, resulting in a 5-year event-free survival (EFS) of 60.0% and OS of 72.7% (Table 1, Figure 1).

3.2 | Metastasis

Forty of 55 patients (72.7%) had pulmonary-only metastasis. Nine patients had pulmonary metastasis and at an additional site (four liver, one bone, two extra-abdominal, one other site, one abdomen), three patients had extensive metastasis in three or more sites including lung and liver, two patients had isolated locoregional peritoneal spread, and one patient isolated liver metastasis (Table 2).

Twenty-four of 52 patients with available data achieved a metastatic CR after pCHT and surgery, and 28 (53.8%) had a non-CR (remission status was not available for three). The remission rate varied according to the site of metastasis (Table 2). Twenty-two out of 39 patients with pulmonary-only metastasis achieved remission after pCHT and surgery, whereas only one out of eight patients with pulmonary metastasis and metastasis at one additional site achieved CR (Table 2).

3.3 | Molecular Genetics and Biomarker

All patients had samples examined by MLPA for the previously selected loci; 1q+ was the commonest CNV, found in 21 (38.2%) patient samples, followed by *MYCN* gain ($n = 10$, 18.2%) and *TP53* loss ($n = 9$, 16.4%). Loss of 16q, loss of 1p, and simultaneous loss of 1p and 16q were present in eight (14.5%), five (9.1%), and one patient (1.8%), respectively (Tables 1 and 3). Loss of *WT1* and *WTX* were detected in two and one patients, respectively. Tumors harboring 1q+ were more likely to have additional CNVs than tumors with 1q other status ($p = 0.03$). In particular, *MYCN* gain occurred significantly more frequently, if 1q+ was present ($p = 0.03$) (Table 3).

1q+ and *TP53* loss were analyzed regarding their respective association with patient characteristics at diagnosis, TV, histology, and outcome. Patients with tumors harboring 1q+ or *TP53* loss were significantly older at diagnosis than patients with 1q and *TP53* ther status ($p = 0.03$ and 0.02, respectively).

Tumors with 1q+ showed a significantly worse 5-year EFS (EFS: 42.9% for patients with 1q+ tumors vs. 70.6% 1q other status, $p = 0.04$), which did however not translate in a significant inferior OS (Table 1, Figure 1, $p = 0.19$). Of the 18 patients with tumors harboring 1q+, pulmonary metastasis ($n = 15$ with pulmonary-only metastasis) and complete data on RT, 13 had no pRT (seven events) and five had pRT (two events).

Tumors with *TP53* loss showed no significant difference in pCHT-induced volume reduction compared to tumors with *TP53* other status (Table 4), but they were associated with a significantly higher proportion of HR histology ($p < 0.01$) and poorer EFS and OS ($p < 0.01$ for both) than tumors with *TP53* other status (Table 4, Figure 1).

TABLE 1 | Overview.

	Age [months], ($n = 55$)		Sex	N	%	Tumor site	N	%
Patients	Median (IQR)	54 (35–65)	Males	28	50.9	Right	27	49.1
			Females	27	49.1	Left	28	50.9
	At diagnosis [mL] ($n = 50$)		**At surgery [mL] ($N = 46$)**			**Reduction [%] ($N = 46$)**		
TV	Median (IQR)	720 (504–985)	Median (IQR)	175 (76–348)		Median (IQR)	78 (57–86)	
	Risk group	N	%	**Stage**	N	%		
Histology	LR	0	0.0	I	14	25.5		
	IR	44	80.0	II	11	20.0		
	HR	11	20.0	III	30	54.5		
	Preop ($n = 54$)	N	%	**Post-op ($n = 52$)**	N	%	**Remission of metastasis after surgery ($n = 51$)**	
CHT	AV	3	5.6	AVD	29	55.8	CR with CHT	20	38.5
	AVD[a]	50	92.6	VCCD	19	36.5	CR with CHT+OP	4	7.7
	Other	1	1.9	Other	4	7.7	Non-CR	28	53.8
	NA	1		NA	3		NA	3	
	1q status	N	%	**TP53**	N	%	**MYCN**	N	%
Biology	Gain	21	38.2	Loss	9	16.4	Gain	10	18.2
	Normal	34	61.8	Other	46	83.6	Normal	45	81.8
	OS	N	%	**EFS**	N	%	**Tumor specific survival**	N	%
Outcome	Alive	40	72.7	Event free	33	60.0	Alive/Death from other cause	41	74.5
	Dead	15	27.3	Event	22	40.0	Died of cancer	14	25.5

Abbreviations: CHT, chemotherapy; CR, complete remission; EFS, event-free survival; HR, high risk; IR, intermediate risk; N, absolute number of patients with available data; NA, not available; OS, overall survival; TV, tumor volume.

[a]Modified AVD in $N = 25$ patients meaning a dose reduction for any of the three compounds (physician's choice)

No influence of 1q+ and TP53 loss on the remission status after pCHT and surgery was identified. However, regarding *TP53*, only 22.2% of patients with *TP53* loss achieved CR compared to 51.2% with *TP53* other status ($p = 0.15$, Table 4).

Five-year EFS and OS of patients with pulmonary-only metastasis in non-CR after 6 weeks of pCHT±metastatectomy, were significantly worse if 1q+ was present (28.6% [$p = 0.04$] and 38.1% [$p = 0.03$], respectively). For patients in CR, no significant differences were observed for EFS and OS depending on 1q status (Figure 2). We further investigated the prognostic impact of pRT in patients with pulmonary-only metastasis and 1q+. Out of 15 patients with pulmonary-only metastasis and 1q+, three received pRT. No significant differences in 5-year EFS (pRT given: 66.7%, no pRT given: 66.7%, $p = 0.56$) and 5-year OS (66.7% for both pRT given, and no pRT given: 66.7%, $p = 0.85$) were obtained.

4 | Discussion

It has been established that genomic CNVs such as 1q+, *TP53* loss, and *MYCN* gain may affect the malignant potential, response to treatment, and ultimately the prognosis of WT [27, 28, 31, 40–42]. Loss of 16q was also related to an increased risk of relapse and death in the UKW 1–3 trial [32]. The COG identified loss of heterozygosity (LOH) on chromosomes 1p and 16q in WT as an adverse prognostic marker, and is currently used for treatment stratification [13, 40, 43–47]. 1q+ has been identified as a prognostic marker in various series [27, 31, 40, 43, 46–48].

Stage IV WT patients have a worse outcome compared to patients with localized disease, and for those with HR histology the outcome is dismal [6]. Although targeted treatment options and novel agents are currently lacking for WT, these patients could benefit from histology or molecular risk-tailored treatment

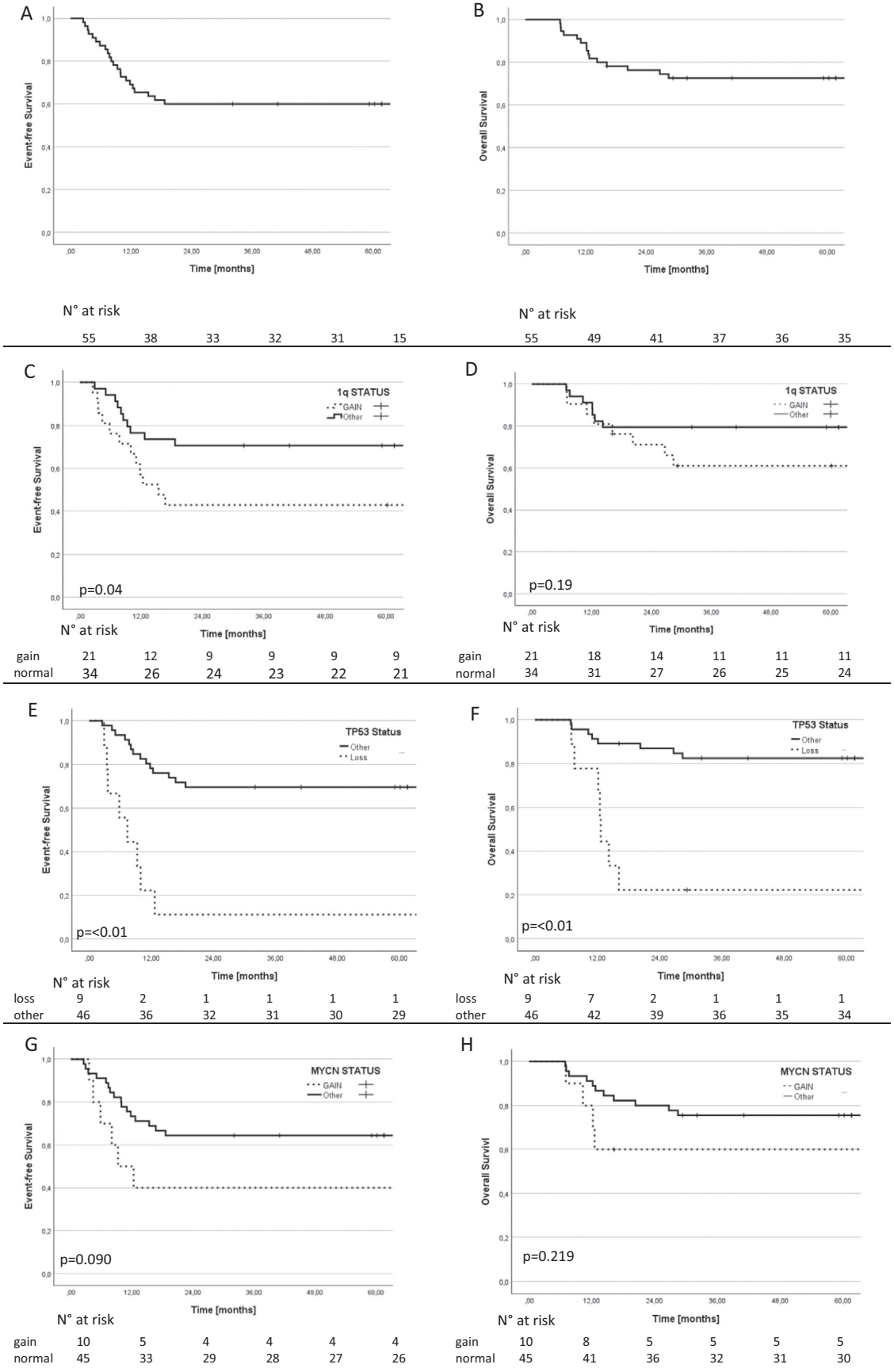


FIGURE 1 | Prognostic impact of the respective copy number variations (CNVs). A and B: all patients ($n = 55$).

TABLE 2 | Site of metastasis and metastatic response.

	Lung only *n* (%)	Lung + 1 other site *n* (%)	Lung, liver + other site *n* (%)	Abdomen *n* (%)	Liver *n* (%)	Total *n* (%)
All						
All	40 (72.7)	9 (16.4)	3 (5.5)	2 (3.6)	1 (1.8)	55 (100)
CR with CHT	18 (46.2)	1 (12.5)			1 (100)	20 (38.5)
CR with surgery	4 (10.3)	0 (0.0)				4 (7.7)
Non-CR	17 (43.6)	7 (87.5)	3 (100)	1 (100)		28 (53.8)
NA	1	1		1		3
1q						
Gain	17 (81.0)	4 (19.0)	0 (0.0)	0 (0.0)	0 (0.0)	21 (100)
CR with CHT	9 (52.9)	1 (25.0)				10 (47.6)
CR with surgery	1 (5.9)	0 (0.0)				1 (4.8)
Non-CR	7 (41.2)	3 (75.0)				10 (47.6)
No gain	23 (67.6)	5 (14.7)	3 (8.8)	2 (5.9)	1 (2.9)	34 (100)
CR with CHT	9 (40.9)				1 (100)	10 (32.3)
CR with surgery	3 (13.6)					3 (9.7)
Non-CR	10 (45.5)	4 (100)	3 (100)	1 (100)		18 (58.1)
NA	1	1		1		3
TP53						
Loss	6 (66.7)	2 (22.2)	0 (0.0)	1 (11.1)	0 (0.0)	9 (100)
CR with CHT	1 (16.7)					1 (11.1)
CR with surgery	1 (16.7)					1 (11.1)
Non-CR	4 (66.7)	2 (100)		1 (100)		7 (77.8)
No loss	34 (73.9)	7 (15.2)	3 (6.5)	1 (2.2)	1 (2.2)	46 (100)
CR with CHT	17 (51.5)	1 (16.7)			1 (100)	19 (44.2)
CR with surgery	3 (9.1)					3 (7.0)
Non-CR	13 (39.4)	5 (83.3)	3 (100)			21 (48.8)
NA	1	1		1 (100)		3
1q and/or TP53						
No alteration	20 (71.4)	4 (14.3)	2 (7.1)	1 (3.6)	1 (3.6)	28 (100)
CR with CHT	9 (47.4)				1 (100)	10 (40.0)
CR with surgery	2 (10.5)					2 (8.0)
Non-CR	8 (42.1)	3 (100)	2 (100)			13 (52.0)
NA	1	1		1		3

Abbreviations: CHT, chemotherapy; CR, complete remission; NA, not available.

[6, 12, 14, 17, 49]. The aim of our study was to determine the clinical and prognostic impact of CNVs in a subgroup of preoperatively treated stage IV WT patients and to assess their suitability for future risk stratification.

A total of 38.2% of patients diagnosed with stage IV WT had 1q+ in their tumors, confirming the relatively high prevalence of 1q+ reported in other metastatic WT series [27, 31, 48, 50]. Previous analyses of unilateral WT patients of all stages found 1q+ in 28% and 19% of patients, respectively [27, 31], and the COG reported a prevalence of 28% of 1q+ in a large cohort of WT with favorable histology (stage II–IV), rising to 44% if only stage IV is considered [46]. This suggests that 1q+ is more common in advanced WT stages, in line with observations from Hing et al. [43]. In our cohort, 1q+ occurred in 38.2% of patients and was associated with a significantly higher age at diagnosis than 1q other status (median age at diagnosis 65 months vs. 49 months). 1q+ had no impact on the TV at diagnosis ($p = 0.46$, Table 4).

For WT of all stages, SIOP-RSTG and COG reported significantly worse EFS for 1q+ [27, 31, 46]. The higher relapse risk was described earlier in independent patient cohorts from different

TABLE 3 | 1q gain and corresponding copy number variations.

		1q status		
		Gain ($n = 21$)	**Other ($n = 34$)**	***p***
		***n* (%)**	***n* (%)**	
1p status	Loss ($n = 5$)	3 (14.3)	2 (5.9)	0.36[a]
	Other ($n = 50$)	18 (85.7)	32 (94.1)	
16q status	Loss ($n = 8$)	2 (9.5)	6 (17.6)	0.70[a]
	Other ($n = 47$)	19 (90.5)	28 (82.4)	
1p16q status	Loss ($n = 1$)	0 (0.0)	1 (2.9)	>0.99[a]
	Other ($n = 54$)	21 (100.0)	33 (97.1)	
MYCN status	Gain ($n = 10$)	7 (33.3)	3 (8.8)	0.03[a]
	Other ($n = 45$)	14 (66.7)	31 (91.2)	
TP53 status	Loss ($n = 9$)	4 (19.0)	5 (14.7)	0.72
	Other ($n = 46$)	17 (81.0)	29 (85.3)	
WT1 status	Loss ($n = 2$)	1 (4.8)	1 (2.9)	0.73[a]
	Other ($n = 53$)	20 (95.2)	33 (97.1)	
WTX status	Loss ($n = 1$)	1 (4.8)	0 (0.0)	0.38[a]
	Normal ($n = 54$)	20 (95.2)	34 (100.0)	
Overall CNV status[c]	Variation ($n = 26$)	14 (66.7)	12 (35.3)	0.03[b]
	Neutral ($n = 29$)	7 (33.3)	22 (64.7)	

Note: "Other" includes copy number variation (CNV) normal status, and gains or losses dependent on the respective variable.

[a]Fisher's exact test.

[b]Chi-square test.

[c]All CNVs of the left column included.

research groups, and seems particularly important in stage IV patients [40, 43, 46, 48, 51, 52].

Given this negative prognostic potential, we analyzed our data for a correlation of 1q+ with established prognostic factors such as histology, TV, and remission status of metastasis at the time of surgery [3, 7, 21]. In tumors with 1q+, a nonsignificant higher proportion of HR histology was observed, which is in line with the described association of 1q+ and HR blastemal subtype after pCHT [27, 53].

The COG reported for patients with only pulmonary metastasis and favorable histology, a remission rate after 6 weeks of CHT of 33.3% when 1q+ was present, compared to 50.3% for 1q other status [47]. In our series, 1q+ did not affect the remission rate of metastasis after pCHT ± metastasectomy, and TVs pre-nephrectomy did not differ from tumors with 1q other status. Patients with pulmonary-only metastasis had significant inferior EFS and OS if they were not in metastatic CR after pCHT ± metastasectomy and 1q+ was present in their tumors. For patients achieving CR after pCHT, no significant differences in EFS and OS were found depending on 1q status (Figure 2). This is in contrast with the results from the COG that reported for patients with 1q+ and pulmonary-only metastasis having achieved CR after 6 weeks of chemotherapy, a significantly worse EFS and trend toward inferior OS, but this was interestingly not observed in patients with non-CR [47]. This was explained by the omission of pulmonary RT in the subclass of patients with CR. In our small series, administration of pRT in patients with pulmonary-only metastasis and 1q+ did not significantly improve 5-year EFS or OS (only 15 patients with available data, of whom only three received pRT). However, it must be considered that our cohort included patients with HR histology composed of DA only, whereas the COG cohort only comprised patients with favorable histology. Additionally, pulmonary CR was defined differently. In our cohort, it could be achieved by either pCHT or metastasectomy, whereas in the COG cohort, CR by chemotherapy only was required. Nevertheless, in summary, our results together with those of the COG emphasize the negative prognostic impact of 1q+ and suggest a potential benefit of treatment intensification.

As current protocols only consider treatment intensification in case of HR histology or preoperative non-CR, the question arises whether IR patients with 1q+ WT should also benefit from postoperative treatment intensification (including RT to the metastatic sites). This needs to be determined in further prospective clinical studies.

Concurrent *MYCN* gain was found in a significant higher proportion in tumors with 1q+. The relation between 1q+ and simultaneous loss of 1p and 16q could not be assessed in our series due to small numbers (Table 3). However, in general, additional CNVs were significantly more frequent in tumors with 1q+ than in tumors with 1q other status, suggesting a more

TABLE 4 | Clinical impact of 1q gain and TP53 loss.

	1q status			*TP53* status			1q and/or *T53* status		
	1q+	***Other***	***p***	***Loss***	***Other***	***p***	***1q+ and/or TP53 loss***	***Other***	***p***
Frequency, *n* (%)									
	21 (38.2)	34 (61.8)		9 (16.4)	46 (83.6)		26 (47.3)	29 (52.7)	
Age [months]									
Median (IQR)	65 (42–75)	49 (28–60)	0.03	64 (57–109)	49 (34–65)	0.02	64 (45–82)	47 (27–60)	0.02
Sex, *n* (%)									
Females	12 (57.1)	15 (44.1)	0.35	7 (77.8)	20 (43.5)	0.08	16 (61.5)	11 (37.9)	0.08
Males	9 (42.9)	19 (55.9)		2 (22.2)	26 (56.5)		10 (38.5)	18 (62.1)	
TV at diagnosis [mL]									
Median (IQR)	612 (306–1074)	734 (535–962)	0.46	667 (420–940)	727 (494–1074)	0.73	747 (323–1085)	703 (520–960)	0.99
TV at surgery [mL]									
Median (IQR)	180 (60–373)	165 (81–380)	0.69	218 (168–929)	161 (55–340)	0.10	189 (87–442)	151 (59–347)	0.33
TV reduction [%]									
Median (IQR)	70 (54–80)	80 (63–89)	0.25	54 (8–82)	78 (59–89)	0.06	75 (44–80)	83 (64–90)	0.15
Abdominal stage, *n* (%)									
I	2 (9.5)	12 (35.3)	0.11	0 (0.0)	13 (28.8)	0.08	2 (7.7)	11 (37.9)	0.01
II	5 (23.8)	6 (17.6)		1 (11.1)	11 (23.9)		5 (19.2)	7 (24.1)	
III	14 (66.7)	16 (47.1)		8 (88.9)	22 (47:8)		19 (73.1)	11 (37.9)	
Histological subtype, *n* (%)									
IR	16 (76.2)	28 (82.4)	0.73	2 (22.2)	42 (91.3)	<0.01	17 (65.4)	27 (93.1)	0.01
HR	5 (23.8)	6 (17.6)		7 (77.8)	4 (8.7)		9 (34.6)	2 (6.9)	
Remission status after chemotherapy and surgery, *n* (%)									
CR	11 (52.4)	13 (41.9)	0.57	2 (22.2)	22 (51.2)	0.15	12 (46.2)	12 (46.2)	1.00
Non-CR	10 (47.6)	18 (58.1)		7 (77.8)	21 (48.8)		14 (53.8)	14 (53.8)	
NA		3			3			3	
Survival function									
5-year EFS (%)	42.9	70.6	0.04	11.1	69.6	<0.01	37.0	82.1	<0.01
5-year OS (%)	61.0	79.4	0.19	22.2	82.4	<0.01	51.2	92.9	<0.01

Abbreviations: CR, complete remission; EFS, event-free survival; HR, high risk; IR, intermediate risk; NA, not available; OS, overall survival; TV, tumor volume.

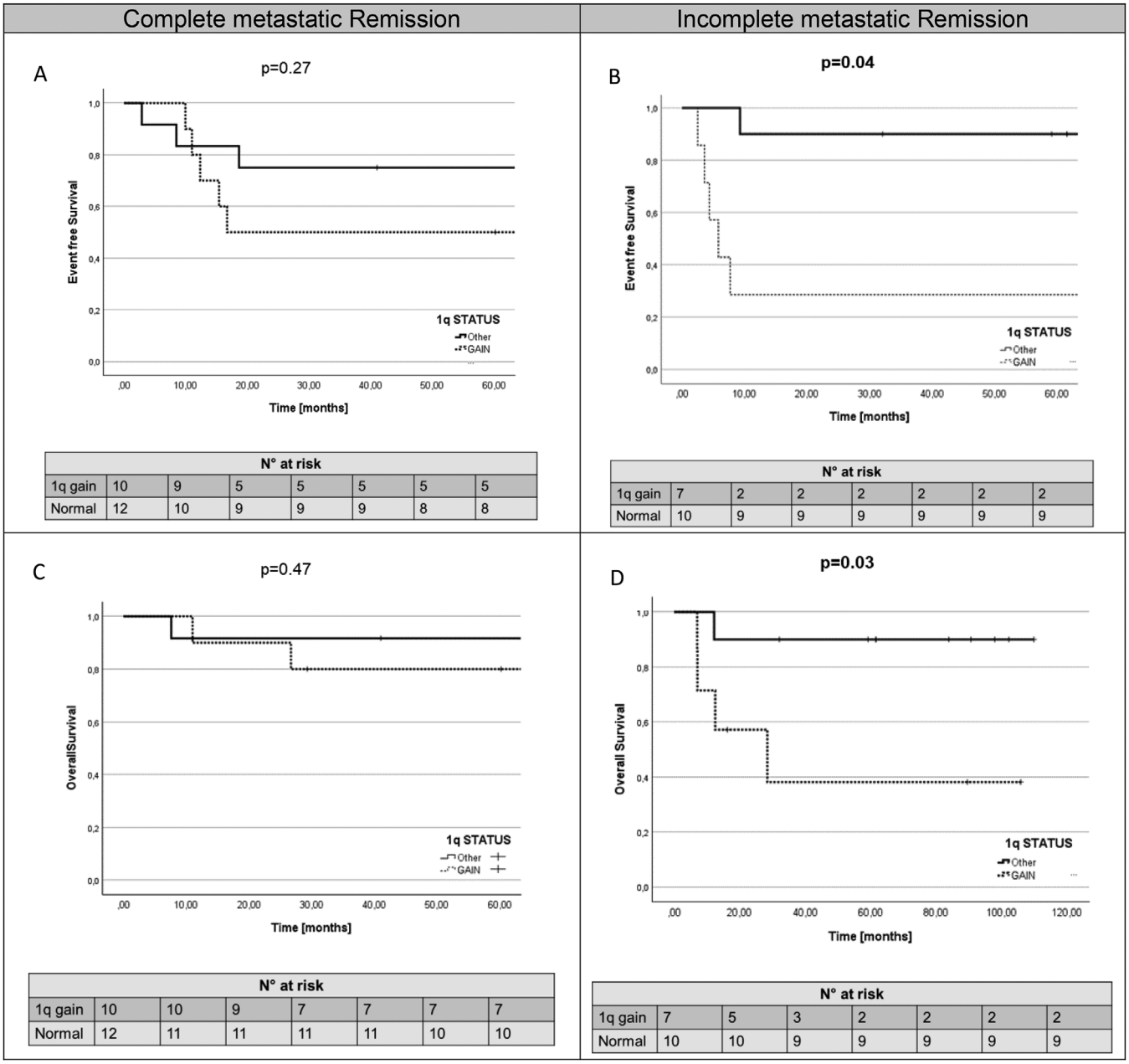
unstable genome when 1q+ is present. Similar observations are described for *TP53* loss [28, 54].

TP53 loss was less common than 1q+ but still occurring in 16.4% of this stage IV cohort. Interestingly, females were more frequently affected (seven out of nine patients, Table 4). Like 1q+, *TP53* loss correlated with a significant older age at diagnosis, which has been related to an unfavorable outcome in previous studies [24, 25].

We confirm the strong but not exclusive association of *TP53* loss with HR histology [36, 41]. Our series of stage IV tumors with *TP53* loss showed a very poor outcome, with a 5-year EFS and OS of only 11.1% and 22.2%, respectively, which is consistent with COG data reporting a particularly poor outcome of patients with stage IV WT, if *TP53* was not wild-type [42].

When assessing the local extent of disease at diagnosis and the response to pCHT, we found no difference in initial TV, but a trend toward less TV reduction in response to pCHT and higher local tumor stages at surgery in tumors with *TP53* loss compared to *TP53* other status (Table 4). Both are described to correlate with poorer outcome [7, 32, 41].

Regarding metastasis, a higher proportion of metastatic non-CR was observed after pCHT in patients having tumors with *TP53* loss (seven out of nine, 77.8%) compared to 48.8% with *TP53*other status (Tables 2 and 4). The association between *TP53* loss and other negative prognostic factors (higher local stage, less preoperative volume reduction, higher rate of non-CR) needs to be further investigated in larger and more representative series in the future. If our results are confirmed, this will emphasize the clinical need of molecular diagnostics to determine, for example, *TP53* status at the time of diagnosis. According to SIOP protocols,



N° at risk							
1q gain	10	9	5	5	5	5	5
Normal	12	10	9	9	9	8	8

N° at risk							
1q gain	7	2	2	2	2	2	2
Normal	10	9	9	9	9	9	9

N° at risk							
1q gain	10	10	9	7	7	7	7
Normal	12	11	11	11	11	10	10

N° at risk							
1q gain	7	5	3	2	2	2	2
Normal	10	10	9	9	9	9	9

FIGURE 2 | Outcome of patients with pulmonary-only metastasis according to remission status after pCHT ± metastatectomy and presence of 1q+.

histology at diagnosis is often not available, and the development of liquid biopsies detecting circulating tumor DNA could be helpful as a diagnostic tool. By using this in the future, treatment could be adapted earlier in specific cases. It has already been shown that the detection of *TP53*-mutated WT in circulating DNA in the patient's blood is possible using a droplet digital polymerase chain reaction [55].

In the current SIOP-RTSG-2016 UMBRELLA protocol, liquid biopsies are performed within a research setting at well-defined time points. In a pilot series, the determination of molecular tumor markers appears to be possible using liquid biopsies [56–59].

MYCN gain, which was associated with adverse outcome in previous WT series [29], was the second most prevalent CNV (18.2% of tumors) in our series. It has been identified as corresponding CNV to 1q+ ($p = 0.03$) and patients with *MYCN* gain had a nearly significant inferior EFS ($p = 0.09$) (Tables 1 and 3, Figure 1). Regarding the prevalence of 1p and 16q loss, our results (9.1% and 14.5% of patients, respectively, in our series) are consistent with literature [32]. No difference in frequency was seen in our stage IV patients compared to localized stages [27]. Only one patient (1.8%) had simultaneous loss of 1p and 16q.

Our study has some limitations. A selection bias is likely as only 55 of 373 stage IV patients had sufficiently complete data to be included in the analysis, and only patients from three European countries were selected. The extent to which our cohort is representative of the overall stage IV cohort cannot be accurately assessed and must be investigated in prospective studies. Particularly, the distribution of risk groups of 80% IR

versus 20% HR (all DA) is biased and contributes to a worse outcome of our patient cohort. In addition, our results are based on a small number of patients. Since in SIOP 2001 study, consent for biological studies was not defined as an inclusion criterion no standardized biomarker sampling was performed across all participating countries. Furthermore, not all relevant molecular changes can be determined with copy number analysis. In *TP53* and *MYCN*, single nucleotide variants have a considerable influence on the biology in WT. In case of *MYCN*, P44L point mutation is particularly noteworthy, but it is still not as frequent as *MYCN* gain (e.g., 4.1% vs. 11.5% in Gadd et al., 2017). Thus, our data cover at least a major share of MYCN alterations. Similarly, TP53 point mutations that we did not score here, are often combined with the loss of the second allele, which is detectable by MLPA. This was the case in 91% and 82% of TP53 mutant cases reported by Maschietto et al. and Ooms et al., respectively [29, 42, 60]. Our results are based on a single tumor sampling. However, intratumoral heterogeneity was described for 1q+ in particular, suggesting that not all 1q+ tumors may have been detected in our study [54, 61]. In UMBRELLA, systematic multisampling of blood as well as fresh frozen and viable tumor tissue at protocol-defined time points will be performed to obtain more representative results [62].

In conclusion, our analysis confirms the prognostic significance of 1q+ and *TP53* loss for the specific subgroup of preoperatively treated patients with stage IV WT, which was previously shown for the overall WT cohort of SIOP 2001 by Chagtai et al. In particular, our results underline their potential benefit for future treatment stratification in the stage IV subgroup. Considering the proportion of 76% IR histology in patients with stage IV and 1q+ and their significantly inferior EFS compared to patients without 1q+, the question arises of whether further intensification of postoperative therapy for patients with IR stage IV WT presenting 1q+ is necessary in future studies. The prognostic value of 1q+ for the stage IV subgroup is underlined by significantly worse 5-year EFS and OS of patients with pulmonary-only metastases in non-CR after pCHT and surgery when 1q+ was present. SIOP-RTSG-2016 UMBRELLA and SIOP Randomet 2017 will provide further insights on the prognostic impact of CNVs.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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