European Journal of Cancer 118 (2019) 58–66

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Treatment of stage I anaplastic Wilms' tumour: a report from the Children's Oncology Group AREN0321 study☆,☆☆

Najat C. Daw [a,*], Yueh-Yun Chi [b], Yeonil Kim [b], Elizabeth A. Mullen [c], John A. Kalapurakal [d], Jing Tian [e], Geetika Khanna [f], James I. Geller [g], Elizabeth J. Perlman [h], Peter F. Ehrlich [i], Anne B. Warwick [j], Paul E. Grundy [k], Conrad V. Fernandez [l], Jeffrey S. Dome [m] on behalf of the AREN0321 Study Committee

[a] *Division of Pediatrics, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd Unit 87, Houston, TX 77030-4009, USA*
[b] *Department of Biostatistics, University of Florida, 6011 NW 1st Place Suite 10, Gainesville, FL 32607, USA*
[c] *Department of Pediatric Hematology/Oncology, Dana-Farber/Harvard Cancer Center, Dana Farber Cancer Institute, 450 Brookline Ave. Office#: SW360A, Boston, MA 02215-5450, USA*
[d] *Department of Radiation Oncology, Ann and Robert H. Lurie Children's Hospital of Chicago, Northwestern Memorial Hospital, 25i East Huron Street Suite#L178, Chicago, IL 60611, USA*
[e] *Children's Oncology Group Data Center, Statistics Data Center, 6011 NW 1st Place Suite 10, Gainesville, FL 32607, USA*
[f] *Mallinckrodt Institute of Radiology, Washington University School of Medicine, 510 S. Kingshighway, Box 8131, St. Louis, MO 63110, USA*
[g] *Division of Oncology, Cincinnati Children's Hospital Medical Center, University of Cincinnati, 3333 Burnet Avenue MLC 7015, Cincinnati, OH 45229, USA*
[h] *Department of Pathology, Ann and Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, 225 East Chicago Avenue, Chicago, IL 60611, USA*
[i] *Department of Pediatric Surgery, University of Michigan, 4-945 C.S. Mott Children's Hospital SPC 4211, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-4211, USA*
[j] *Department of Pediatrics, F. Edward Hébert School of Medicine, Uniformed Services University, 4301 Jones Bridge Road, Bethesda, MD 20814, USA*
[k] *Department of Pediatrics, University of Alberta Hospital, WMC 4E2.28, 8440-112 Street, Edmonton, Alberta T6G 2B7, Canada*

☆ This article was presented in part at the 48th Congress of the International Society of Paediatric Oncology, Dublin, Ireland, in October 2016.

☆☆ Research support: The research was supported by the St. Baldrick's Foundation and grants from the National Institutes of Health to the Children's Oncology Group (U10CA180886, U10CA180899, U10CA098543, U10CA098413 and U24CA114766) and the National Wilms Tumor Study Group (NCT00002611). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

* *Corresponding author*: Division of Pediatrics, MD Anderson Cancer Center, Unit 87. 1515 Holcombe Blvd, Houston, TX 77030. Fax: +1 713 792 0608.

E-mail addresses: ndaw@mdanderson.org (N.C. Daw), ychi@cog.ufl.edu (Y.-Y. Chi), elizabeth_mullen@dfci.harvard.edu (E.A. Mullen), j-kalapurakal@northwestern.edu (J.A. Kalapurakal), jtian@cog.ufl.edu (J. Tian), khannag@wustl.edu (G. Khanna), james.geller@cchmc.org (J.I. Geller), eperlman@luriechildrens.org (E.J. Perlman), pehrlich@med.umich.edu (P.F. Ehrlich), anne.warwick@usuhs.edu (A.B. Warwick), paul.e.grundy@ahs.ca (P.E. Grundy), conrad.fernandez@iwk.nshealth.ca (C.V. Fernandez), jdome@childrensnational.org (J.S. Dome).

https://doi.org/10.1016/j.ejca.2019.05.033

[l] Departments of Pediatrics and Bioethics, IWK Health Centre, Dalhousie University and the IWK Health Centre, 5850 University Avenue, Halifax, Nova Scotia B3K 6R8, Canada
[m] Division of Oncology, Children's National Medical Center, Center for Cancer and Blood Disorders, George Washington University School of Medicine and Health Sciences, 111 Michigan Avenue NW, Washington, DC 20010, USA

Received 27 January 2019; received in revised form 30 March 2019; accepted 22 May 2019
Available online 17 July 2019

KEYWORDS
Wilms tumour;
Stage I;
Diffuse anaplasia;
Focal anaplasia;
Treatment;
Vincristine;
Dactinomycin;
Doxorubicin;
Radiation;
Outcome

Abstract ***Background:*** In the fifth National Wilms Tumor Study (NWTS-5), the 4-year event-free survival (EFS) and overall survival (OS) estimates for 29 patients with stage I focal (n = 10) or diffuse (n = 19) anaplastic Wilms' tumour (AWT) treated with vincristine and dactinomycin without flank radiation were 69.5% and 82.6%, respectively. The Children's Oncology Group AREN0321 study evaluated whether adding doxorubicin and flank radiation improves survival for these patients.
Patients and methods: Tumour histology and stage were confirmed by real-time central pathology, surgery and radiology review. The patients received 25 weeks of vincristine, dactinomycin and doxorubicin (cumulative dose 150 mg/m^2) with flank radiation (1080 cGy). We retrospectively analysed outcomes of all patients with stage I AWT enrolled in NWTSs 1–5 and AREN0321 with respect to treatment regimens.
Results: Eighteen patients with stage I AWT (8 focal and 10 diffuse) were enrolled on AREN0321. With a median follow-up of 4.6 years, the 4-year EFS and OS were 100%. One patient with diffuse AWT had pulmonary relapse 4.12 years after diagnosis. In the 112 patients with stage I AWT treated in NWTSs 1–5 and AREN0321, the EFS was significantly improved with doxorubicin treatment (p = 0.01; 4-year EFS: 97.2% [95% confidence interval {CI}: 91.3–100] vs. 77.5% [95% CI: 67.6–87.4]) but not by flank radiation (p = 0.15).
Conclusions: Treatment of stage I AWT with vincristine, dactinomycin, doxorubicin and flank radiation in AREN0321 yielded excellent survival outcomes. Retrospective analysis of AREN0321 and NWTS patients suggests that doxorubicin had a greater contribution to the excellent outcomes than radiation.

1. Introduction

Approximately 5–10% of Wilms' tumours demonstrate anaplastic histology, which is defined by the presence of polyploid atypical mitotic figures, a large nuclear size and hyperchromasia [1,2]. The definition of anaplasia is further refined to specify whether the anaplasia is diffuse or focal based on the geographic distribution of anaplastic cells within the tumour [3]. The presence of anaplasia is one of the most powerful adverse prognostic factors for Wilms' tumour. Wilms' tumours with diffuse anaplasia are associated with inferior event-free survival (EFS) and overall survival (OS) estimates compared with tumours of favourable histology; tumours with focal anaplasia have an intermediate prognosis. The third National Wilms Tumor Study (NWTS-3) was the first study to augment therapy for anaplastic Wilms' tumour, with outcomes improving with the addition of cyclophosphamide, and later etoposide, to treatment regimens [4,5].

In NWTS-5, patients with stage I diffuse or focal anaplastic Wilms' tumour were treated with vincristine and dactinomycin without flank radiation (Regimen EE4A), the same regimen used for favourable histology Wilms' tumour. The rationale for this approach was that previous studies have shown relatively favourable outcomes for stage I anaplastic Wilms' tumour, although some of these studies included combinations of doxorubicin, cyclophosphamide and/or flank radiation [6,7]. Moreover, because stage I tumours are confined to the kidney and completely resected, it was thought that all the anaplastic cells are removed and additional therapy is not required. However, among patients with stage I focal and diffuse anaplasia enrolled in NWTS-5, the 4-year EFS and OS estimates were only 69.5% (95% confidence interval [CI], 46.9–84.0) and 82.6% (95% CI, 63.1–92.4), respectively. By contrast, the 4-year EFS and OS estimates for 473 patients with stage I favourable histology Wilms' tumour were 92.4% (95% CI, 89.5–94.5) and 98.3% (95% CI, 96.4–99.2), respectively [5]. The sites of relapse among the patients with stage I anaplastic Wilms' tumour included the lung (1), operative bed (1), abdomen and pelvis outside the operative bed (2), liver (2) and other sites (2).

To improve on these results, the Children's Oncology Group (COG) AREN0321 study evaluated the treatment of patients with stage I anaplastic Wilms' tumour with vincristine, dactinomycin, doxorubicin (Regimen DD4A) and flank radiation. Here, we report the outcomes of patients treated with this approach and the results of a retrospective analysis of all patients with stage I anaplastic Wilms' tumour treated in NWTSs 1–5 and COG AREN0321 to identify treatment factors that influenced outcomes.

2. Patients and methods

The AREN0321 study (ClinicalTrials.gov identifier: NCT00335556) was approved by the National Cancer Institute Pediatric Central Institutional Review Board (IRB) and local IRBs according to institutional policy. The NWTSs 1–5 were approved by the local IRBs. All participants or their legally authorised guardians provided consent.

2.1. Patients

Eligible patients had stage I focal or diffuse anaplastic Wilms' tumour. All patients underwent upfront nephrectomy and began protocol therapy no later than day 14 after nephrectomy. The standard operation was a unilateral radical nephroureterectomy with lymph node sampling. Expert central review of a complete set of pathology slides, the pathology reports, surgical summaries and protocol required imaging studies was performed before enrollment in AREN0321 through mandatory enrollment on the COG AREN03B2 biology and classification protocol. Stage I disease was defined according to the following criteria: (1) the tumour is limited to the kidney and is completely resected, (2) the renal capsule is intact, (3) the tumour was not ruptured or biopsied before removal, (4) the vessels of the renal sinus are not involved, (5) there is no evidence of tumour at or beyond the margins of resection and (6) regional lymph nodes that were examined microscopically were free of tumour.

Patients had to be younger than 30 years, have a Karnofsky or Lansky performance status of ≥50, have not received systemic chemotherapy or radiation, have adequate cardiac function defined as shortening fraction ≥27% by echocardiography or ejection fraction ≥50% by radionuclide angiography and have adequate liver function defined as total serum bilirubin ≤1.5 × normal for age and liver transaminases

Week																								
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
V	V	V	V	V	V	V	V	V	V			V‡			V‡			V‡			V‡			V‡
A			D+			A			D+			A			D			A			D			A

Radiation therapy to begin at week 1

V	Vincristine	0.025 mg/kg/day IV x 1 for infants < 1 yr 0.05 mg/kg/day IV x 1 for children 1 yr–2.99 yr 1.5 mg/m^2/day IV x 1 for children ≥ 3 yrs	Maximum dose: 2 mg
V‡	Vincristine	0.034 mg/kg/day IV x 1 for infants < 1 yr 0.067 mg/kg/day IV x 1 for children 1 yr–2.99 yr 2 mg/m^2/day IV x 1 for children ≥ 3 yr	Maximum dose: 2 mg
A	Dactinomycin	0.023 mg/kg/day IV x 1 for infants < 1 yr 0.045 mg/kg/day IV x 1 for children ≥ 1 yr	Maximum dose: 2.3 mg
D+	Doxorubicin	1.5 mg/kg/day IV x 1 for infants < 1 yr 45 mg/m^2/day IV x 1 for children ≥ 1 yr	
D	Doxorubicin	1 mg/kg/day IV x 1 for infants < 1 yr 30 mg/m^2/day IV x 1 for children ≥ 1 yr	

Fig. 1. Treatment schema for Regimen DD4A and chemotherapy dosing.

<2.5 × normal for age. Female patients of childbearing age had to have a negative pregnancy test and, if lactating, had to stop breastfeeding. Sexually active patients of childbearing potential had to use effective contraception.

2.2. Protocol therapy

Patients received Regimen DD4A with vincristine, doxorubicin, dactinomycin and radiation to the flank (Fig. 1). An absolute neutrophil count of ≥750/μL and platelet count of ≥75,000/μL were required to start myelosuppressive chemotherapy. Vincristine was held until peristalsis was established after nephrectomy and was continued during the weekly schedule, regardless of blood counts. Radiation therapy (10.8 Gy) to the flank was delivered as previously described, concurrently with initiation of chemotherapy by day 14 after nephrectomy [8,9]. Radiation therapy was delivered at COG-approved centres. The detailed treatment plan and dosimetry were required to be submitted to the Quality Assurance Review Center for review.

2.3. Patient evaluation

Laboratory testing, computed tomography (CT) of the chest, abdomen and pelvis, electrocardiography and echocardiography were performed at baseline and the end of therapy. The laboratory tests included complete blood counts, determination of serum creatinine, alanine aminotransferase, bilirubin, electrolytes, calcium, phosphorus, magnesium and total protein/albumin and urinalysis. During therapy, an echocardiogram was repeated before week 16. After completion of the therapy, the patients were followed up for disease surveillance every 3 months during the first 2 years, every 6 months during the third and fourth years and every 12 months during the fifth year. CT of the chest, abdomen and pelvis was obtained at every visit during the first 2 years and switched to chest radiography and abdominal ultrasonography during the subsequent years.

2.4. Retrospective analysis of data of NWTSs 1–5

The results of NWTSs 1–5 were previously reported [1,5–7,10]. Patients with stage I diffuse or focal anaplastic Wilms' tumour were identified using the NWTS database. Patients who were confirmed to have stage I disease and anaplastic histology by retrospective central pathology and a study chair review were included in the analysis. The treatment regimens used on NWTSs 1–5 are summarised in Table 1.

2.5. Definitions of focal and diffuse anaplasia

Focal anaplasia was defined as the presence of one or a few sharply localised regions of anaplasia within a primary tumour, the majority of which do not contain nuclear atypia. The topographic definition of focal anaplasia required careful documentation of the exact site, from which every section was obtained (e.g. on a diagram, specimen photocopy and/or polaroid photograph of the gross specimen). Diffuse anaplasia was defined as (1) anaplasia in any extrarenal site, including vessels of the renal sinus, extracapsular infiltrates, nodal or distant metastases; (2) anaplasia in a random biopsy specimen; (3) anaplasia unequivocally expressed in one region of the tumour but with extreme nuclear pleomorphism approaching the level of anaplasia elsewhere in the tumour and (4) anaplasia in more than one tumour slide unless (a) every slide showing anaplasia came from the same region of the tumour or (b)

Table 1
Treatment regimens used for patients with stage I anaplastic histology Wilms' tumour enrolled in NWTSs 1–5.

Study	Regimen name	Chemotherapy regimen	XRT	No. of patients (FA/DA)
NWTS-1	A	AMD × 15 mo	Yes	0/2
	B	AMD × 15 mo	No	0/2
NWTS-2	C	AMD/VCR × 15 mo	Yes	1/0
	D	VCR/AMD/DOX × 15 mo	Yes	0/1
	E	VCR/AMD × 6 mo	No	1/1
	F	VCR/AMD × 15 mo	No	0/4
NWTS-3	DD	VCR/AMD/DOX × 15 mo	No	1/2
	DD-RT/DD2000	VCR/AMD/DOX × 15 mo	Yes	2/8
	EE	VCR/AMD × 25 wk	No	1/4
	J	VCR/AMD/DOX/CTX × 15 mo	Yes	0/4
	L	VCR/AMD × 10 wk	No	2/0
NWTS-4	EE	VCR/AMD-std. × 25 wk	No	8/9
	EE4A	VCR/AMD-PI × 18 wk	No	7/6
	K	VCR/AMD-std × 65 wk	No	0/1
NWTS-5	EE4A	VCR/AMD-PI × 18 wk	No	8/19

NWTS, National Wilms Tumor Study; FA, focal anaplasia; DA, diffuse anaplasia; VCR, vincristine; wk, weeks; mo, months; AMD, dactinomycin; std, standard dosing; PI, pulse-intensive dosing; DOX, doxorubicin; CTX, cyclophosphamide; XRT, radiation therapy.

anaplastic foci on the various slides are small and surrounded on all sides by non-anaplastic tumour.

2.6. Statistical methods

Data from AREN0321 patients who were confirmed to have stage I anaplastic Wilms' tumour on central pathology review were analysed. The clinical characteristics of patients treated in AREN0321 and NWTS-5 were compared using the Wilcoxon rank-sum test for age, chi-square test for gender and histologic subtype and Fisher's exact test for race/ethnicity. Further analyses were conducted including patients treated in NWTSs 1–5 to evaluate the outcomes of patients with stage I anaplastic Wilms' tumours according to treatment with doxorubicin, flank radiation or both and according to the histologic subtype (focal or diffuse). The t-test was used to examine the relationship between tumour weight and tumour relapse. Both EFS and OS were calculated from the time of diagnosis. The definition of an event included relapse, second malignant neoplasm and death of any cause, whichever occurred first. Local relapse was defined as relapse in the operative bed, abdomen outside the operative bed or pelvis; tumour spread to the liver was considered distant metastasis. The 4-year EFS and OS rates were estimated using the method of Kaplan and Meier [11] with CIs estimated by the Peto–Peto method [12]. EFS and OS were compared between the groups using the log-rank test [13]. The median time of follow-up was calculated based on the Kaplan–Meier estimates [14]. Patient follow-up was carried out until 31st December 2015.

3. Results

From June 2006 to November 2013, a total of 18 eligible patients with stage I anaplastic (8 focal and 10 diffuse) Wilms' tumour were treated with Regimen DD4A in AREN0321. This number does not reflect the total number of patients with stage I anaplastic Wilms' tumour enrolled in the AREN03B2 Renal Tumors Classification and Biology Study during this time period because accrual to the AREN0321 protocol was intermittently suspended for data review. The demographics of these 18 patients and the 27 patients with stage I anaplastic Wilms' tumour treated with Regimen EE4A in NWTS-5 are summarised in Table 2.

3.1. Patient outcomes in AREN0321 and NWTS-5

The median follow-up for survivors in AREN0321 and NWTS-5 was 4.6 years and 13.3 years, respectively. Both the 4-year EFS and OS for the 18 patients treated in AREN0321 were 100%. In comparison, the 4-year EFS and OS for 27 patients treated in NWTS-5 in an updated analysis was 70.0% (95% CI, 51.7%–88.2%; $p = 0.057$) and 81.5% (95% CI, 66.1%–96.9%; $p = 0.057$), respectively (Figs. 2 and 3). One patient with diffuse anaplastic Wilms' tumour treated in AREN0321 had a pulmonary relapse 4.12 years after diagnosis and is alive without evidence of disease 3.92 years after relapse. None of the patients treated in AREN0321 died.

3.2. Patient outcomes in AREN0321 and NWTSs 1–5

In view of the excellent survival observed in patients with stage I anaplastic Wilms' tumour with the addition of doxorubicin and flank radiation to vincristine and dactinomycin in AREN0321, we conducted further analyses to assess whether the addition of doxorubicin, radiation or both contributed to the improved outcomes. These analyses included all 112 patients with stage I anaplastic Wilms' tumour confirmed by central

Table 2
Clinical characteristics of 45 patients with stage I anaplastic Wilms' tumours according to the study.

Characteristics	AREN0321	NWTS-5	P-value[a]
	N (%)	N (%)	
Number of patients	18	27	
Median age at diagnosis, years (range)	4.5 (1.4–13.9)	3.8 (1.4–15)	0.60
Gender			0.11
Male	11 (61)	10 (37)	
Female	7 (39)	17 (63)	
Race/ethnicity			0.18
White	15 (83)	19 (70)	
Black	2 (11)	3 (11)	
Hispanic	–	5 (19)	
Unknown	1 (6)	–	
Histologic subtype			0.31
Focal anaplasia	8 (44)	8 (30)	
Diffuse anaplasia	10 (56)	19 (70)	
Lymph nodes sampled			1.00
Yes	16 (89)	24 (89)	
No	2 (11)	3 (11)	

NWTS, National Wilms Tumor Study.
[a] Wilcoxon rank-sum test for age, chi-square test for gender and histologic subtype, and Fisher's exact test for race/ethnicity.

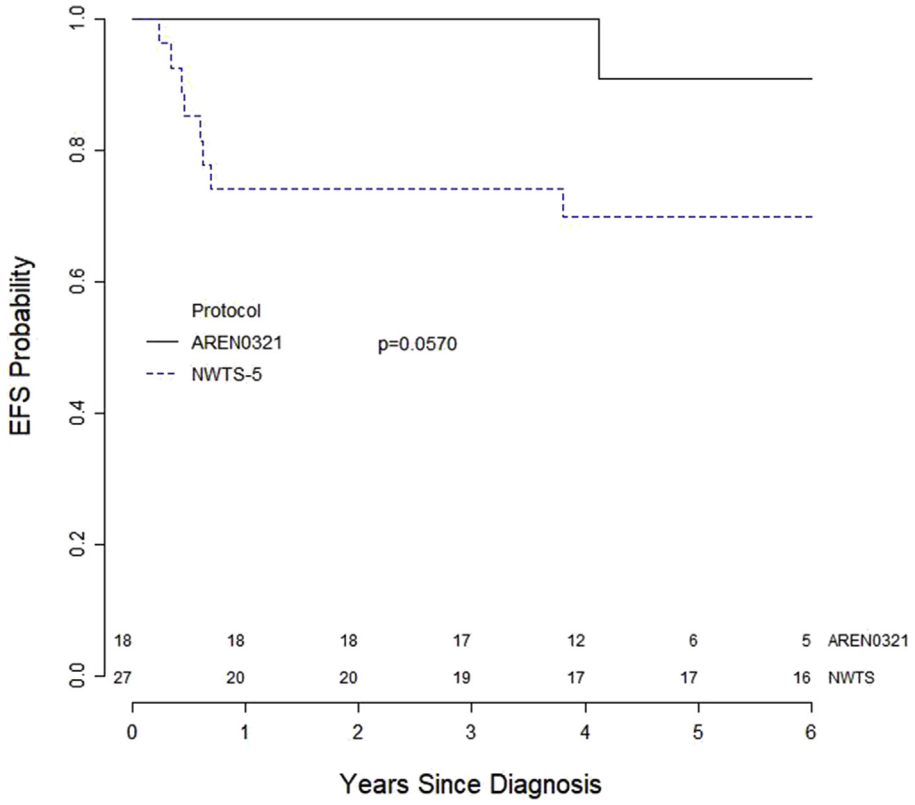

Fig. 2. Event-free survival curves for 45 patients with stage I anaplastic Wilms' tumour according to the study. EFS, event-free survival; NWTS, National Wilms Tumor Study.

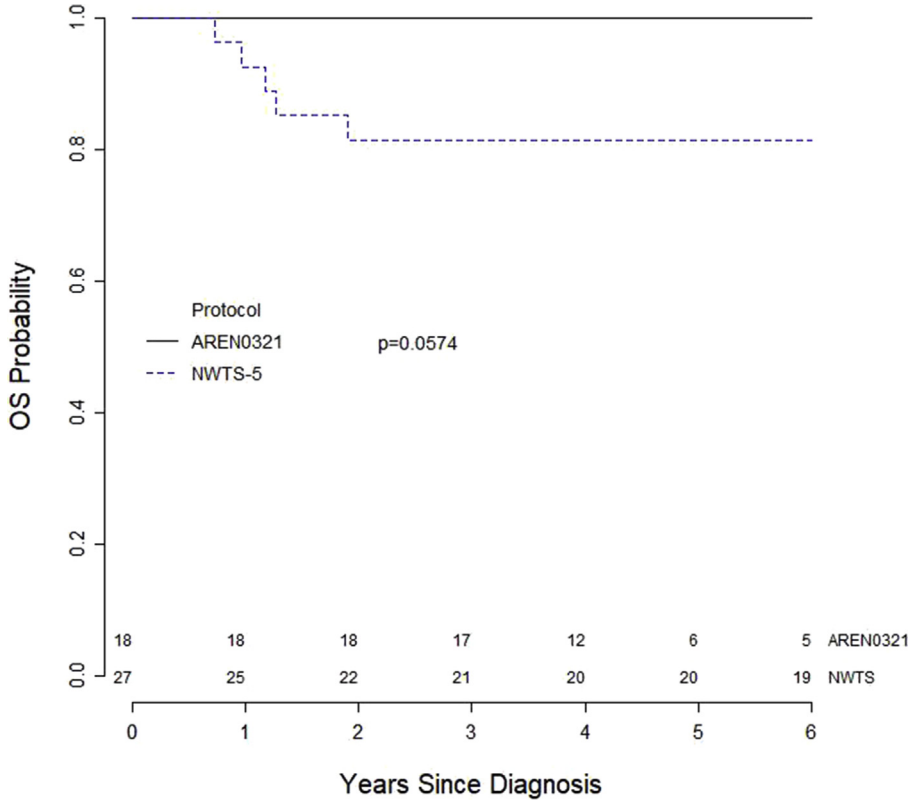

Fig. 3. Overall survival curves for 45 patients with stage I anaplastic Wilms' tumour according to the study. OS, overall survival; NWTS, National Wilms Tumor Study.

Table 3
Analysis of EFS and OS for 112 patients with stage 1 focal or diffuse anaplastic Wilms' tumour treated in AREN0321 and NTWSs 1–5 according to the treatment and histologic subtype.

Factor	Category	N	4-year EFS (95% CI)	P-value[b]	4-year OS (95% CI)	P-value[b]
Overall	–	112	83.8% (76.5–91.1)	–	89.3% (83.1–95.4)	–
Doxorubicin	Yes	36	97.2% (91.3–100)	0.01	97.2% (91.3–100)	0.10
	No	76	77.5% (67.6–87.4)		85.5% (77.2–93.8)	
Radiation	Yes	36	91.7% (81.7–100)	0.15	94.4% (86.2–100)	0.26
	No	76	80.2% (70.7–89.6)		86.8% (78.8–94.8)	
Doxorubicin + radiation[a]	Yes	33	97.0% (90.5–100)	0.03	97.0% (90.5–100)	0.14
	No	73	79.4% (69.6–89.2)		86.2% (77.9–94.5)	
Histology	DA	73	79.5% (69.7–89.2)	0.21	84.9% (76.2–93.5)	0.07
	FA	39	92% (82.7–100)		97.4% (92–100)	

EFS, event-free survival; CI, confidence interval; OS, overall survival; DA, diffuse anaplasia, FA, focal anaplasia.
[a] Excluding 6 patients who received either doxorubicin with no radiation or radiation with no doxorubicin.
[b] Log-rank test.

Table 4
Analysis of EFS and OS for 73 patients with stage I diffuse anaplastic Wilms' tumour according to treatment.

Factor	Category	N	4-year EFS (95% CI)	P-value[b]	4-year OS (95% CI)	P-value[b]
Doxorubicin	Yes	25	96 (88.0–100)	0.046	96% (88.0–100)	0.10
	No	48	70.8 (57.1–84.5)		79% (66.8–91.2)	
Radiation	Yes	25	88.0% (74.6–100)	0.47	92% (80.9–100)	0.33
	No	48	75.0% (62.0–88.0)		81.1% (69.4–92.8)	
Doxorubicin + radiation[a]	Yes	23	95.7% (86.9–100)	0.11	95.7% (86.9–100)	0.16
	No	46	73.9% (60.4–87.4)		80.3% (68.1–92.4)	

EFS, event-free survival; CI, confidence interval; OS, overall survival.
[a] Excluding 4 patients who received either doxorubicin with no radiation or radiation with no doxorubicin.
[b] Log-rank test.

Table 5
Analysis of EFS and OS for 39 patients with stage I focal anaplastic Wilms' tumour according to treatment.

Factor	Category	N	4-year EFS (95% CI)	P-value[b]	4-year OS (95% CI)	P-value[b]
Doxorubicin	Yes	11	100%	0.13	100%	0.47
	No	28	89.1% (77.1–100)		96.4% (89.3–100)	
Radiation	Yes	11	100%	0.06	100%	0.43
	No	28	89.1% (77.1–100)		96.4% (89.3–100)	
Doxorubicin + radiation[a]	Yes	10	100%	0.09	100%	0.47
	No	27	88.7% (76.3–100)		96.3% (88.9–100)	

EFS, event-free survival; CI, confidence interval; OS, overall survival.
[a] Excluding 2 patients who received either doxorubicin with no radiation or radiation with no doxorubicin.
[b] Log-rank test.

pathology review, treated in NWTS-1 (n = 4), NWTS-2 (n = 8), NWTS-3 (n = 24), NWTS-4 (n = 31), NWTS-5 (n = 27) and AREN0321 (n = 18). The 4-year EFS and OS for the 112 patients were 83.8% (95% CI, 76.5–91.1) and 89.3% (95% CI, 83.1–95.4), respectively. The 4-year EFS for patients with stage I diffuse anaplasia was 79.5% (95% CI, 69.7–89.2), compared with 92% (95% CI, 82.7–100) for patients with stage I focal anaplasia (p = 0.21) (Table 3). Sites of relapse among patients with stage I diffuse anaplasia were lung only (n = 8), lung and other sites (n = 1), liver (n = 2), operative bed (n = 2), abdomen outside the operative bed (n = 1), pelvis (n = 1) and other distant sites (n = 2). Sites of relapse among patients with stage I focal anaplasia were lung only (n = 3).

Data on tumour weight were available for 102 patients; of whom, 20 had a relapse and one died after a second malignant neoplasm. The mean tumour weight was 652 g (212–1650 g) for the 21 patients who had a relapse or died vs. 599.9 g (114–2015 g) for the 81 patients without a relapse (p = 0.55).

For the 112 patients with stage I anaplastic histology, treatment with doxorubicin positively impacted EFS. The 4-year EFS for patients who received doxorubicin was 97.2% (95% CI, 91.3–100) and was 77.5% (95% CI, 67.6–87.4) for those who did not (p = 0.01). The 4-year EFS for patients who received doxorubicin plus radiation was 97.0% (95% CI, 90.5–100) and was 79.4% (95% CI, 69.6–89.2) for those who did not (p = 0.030). However, the 4-year EFS did not differ in patients who

received flank radiation (91.7%; 95% CI, 81.7–100) compared with those who did not (80.2%; 95% CI, 70.7–89.6; p = 0.15) (Table 3). Similarly in the subgroup of patients with diffuse anaplastic Wilms' tumour, the 4-year EFS was significantly associated with doxorubicin treatment (p = 0.046) but not with radiation (Table 4). In the subgroup of patients with focal anaplastic Wilms' tumour, there was a trend for better EFS with treatment with radiation (p = 0.06) and with treatment with both doxorubicin and radiation (p = 0.09; Table 5). However, there was near complete overlap between doxorubicin and radiation treatment, making it difficult to separate the effects.

Only four patients (3.6%) had local relapse, all of whom had diffuse anaplasia and lymph node sampling as part of their tumour resection. Among the 73 patients with diffuse anaplasia, one of the 25 (4.0%) patients who received flank radiation and three of the 48 (6.3%) patients who did not receive flank radiation had a local relapse. The one patient with local relapse who received flank radiation and two of the three patients who did not receive flank radiation died. Hence, death from local relapse occurred in 4.0% of patients who received flank radiation and 4.2% of patients who did not.

In terms of OS, there was a trend for improved 4-year OS for patients with focal anaplastic Wilms' tumour (97.4%; 95% CI, 92–100) compared with those with diffuse anaplastic Wilms' tumours (84.9%; 95% CI, 76.2–93.5) (p = 0.07). No statistically significant associations were detected between OS and treatment with doxorubicin, radiation or both (Tables 3–5).

4. Discussion

The AREN0321 study demonstrated that outcomes for patients with stage I anaplastic Wilms' tumour were improved with the addition of doxorubicin and flank radiation to vincristine/dactinomycin therapy. Both 4-year EFS and OS estimates were 100% in AREN0321, compared with 70.0% and 81.5%, respectively, in an updated analysis of NWTS-5. Both doxorubicin and flank radiation were incorporated into the treatment regimen in AREN0321 because the pattern of relapse for stage I anaplastic Wilms' tumour in NWTS-5 included both the abdomen and distant sites. Although these agents increase the risk of long-term side-effects, notably, cardiotoxicity, second malignancies and pregnancy-associated complications, the doses used were relatively low (150 mg/m^2 cumulative doxorubicin dose and 1080 cGy flank radiation). The incremental risks of this therapy must be balanced against the risks of salvage therapy required for recurrence, the psychosocial effects of relapse and the increased likelihood of tumour-related death.

To assess the relative contributions of doxorubicin and flank radiation to the outstanding outcomes seen in AREN0321, we conducted a retrospective analysis of all patients with stage I anaplastic Wilms' tumour treated in the present study and in NWTS 1–5. This review showed a significant improvement in EFS for patients treated with doxorubicin, but no difference in EFS according to flank radiation was shown. The rate of local recurrence was low (3.6%) and appeared to be similar for patients who received flank radiation (4%) and patients who did not receive flank radiation (6.2%). In addition, local relapse occurred only in patients with diffuse anaplasia. These findings suggest that flank radiation may not be necessary for treatment of patients with stage I focal anaplastic Wilms' tumour and raise the question of whether the possible benefit of avoiding a few local relapses is worth the risk of administering relatively low-dose flank radiation to all patients with stage I diffuse anaplastic Wilms' tumour. An international collaborative effort with a larger number of patients of this rare subtype of Wilms' tumour is needed to answer this question definitively.

Other groups have reported outcomes for patients with stage I anaplastic Wilms' tumor, although stage I in the setting of preoperative chemotherapy may have different prognostic implications from stage I in the setting of immediate nephrectomy. Among patients with stage I anaplastic Wilms' tumour treated in the International Society of Pediatric Oncology (SIOP)–6 and SIOP-9 trials, one of the 7 patients with focal anaplasia had tumour recurrence and none died, whereas five of 16 patients with diffuse anaplasia had tumour recurrence and four died [15]. In the subsequent trial SIOP 93-01, the 5-year EFS and OS for patients with stage I intermediate-risk and anaplastic Wilms' tumour treated with only vincristine and dactinomycin were 87% and 95%, respectively, in patients who received only four weeks of postoperative chemotherapy, but the difference in outcome between non-anaplastic and anaplastic tumours was not specified [16].

It would ideal to identify biological prognostic factors that could more precisely select patients who require additional therapy. A strong correlation between *TP53* mutations and anaplasia has been established, and several lines of evidence indicate that such mutations occur as a secondary event in the progression of Wilms' tumour from favourable to anaplastic histology [17–19]. Recent studies have assessed the prognostic significance of the *TP53* mutation or loss of its chromosomal locus at 17p13 in patients with anaplastic Wilms' tumour. Although tumour-specific *TP53* alterations were associated with increased risk of recurrence and death [20], the marker was predictive of adverse outcomes only for advanced stage disease (stage III and IV) [21]. In the latter analysis, a comprehensive assessment of *TP53* alterations including sequencing, copy number analysis and immunohistochemistry demonstrated that the vast majority of anaplastic Wilms' tumours had evidence of *TP53* alterations. Hence, the ability to detect a *TP53* mutation in a random frozen tumour was likely

a surrogate for the overall extent of *TP53* mutations in the tumour, in turn reflecting the burden of anaplasia. Presently, assessment of *TP53* mutational status is not helpful in stratifying therapy for patients with stage I anaplastic Wilms' tumour.

Strengths of the AREN0321 study are that it was a prospective trial for the evaluation of the effect of augmenting therapy for stage I anaplastic Wilms' tumour and that all patients underwent central review of pathology slides, surgical reports and chest CT scans. The study population was an accurately defined and well-annotated group. A caveat to the study is the small number of patients. The retrospective analysis of the 112 patients treated in NWTSs 1–5 and COG AREN0321 has helped to elucidate the contribution of doxorubicin versus flank radiation to the excellent patient outcome. However, the retrospective analysis is limited by the inclusion of patients treated over a broad time frame during which the quality of diagnostic imaging improved and the criteria for stage I changed, as did dosing schemas and duration of chemotherapy regimens.

In summary, EFS for patients with stage I anaplastic Wilms' tumour appears to be improved in AREN0321 compared with NWTS-5 with the addition of doxorubicin and flank radiation to vincristine and dactinomycin. The retrospective analysis of the larger group of patients with stage I anaplastic Wilms' tumour enrolled in the NWTS 1–5 and COG AREN0321 studies indicates that doxorubicin had a greater contribution to the outstanding outcomes than flank radiation. Our study provides important information that will help guide physicians in the treatment of stage I anaplastic Wilms' tumour.

Acknowledgments

The authors wish to thank the parents and children who enrolled in the Wilms' tumour studies and the many investigators, pediatric oncologists, pathologists, surgeons, radiation oncologists, diagnostic imagers and other health professionals who managed the children enrolled in these studies.

Conflict of interest statement

None declared.

References

[1] Beckwith JB, Palmer NF. Histopathology and prognosis of wilms tumors: results from the first national wilms' tumor study. Cancer 1978;41:1937–48.
[2] Zuppan CW, Beckwith JB, Luckey DW. Anaplasia in unilateral wilms' tumor: a report from the national wilms' tumor study pathology center. Hum Pathol 1988;19:1199–209.
[3] Faria P, Beckwith JB, Mishra K, Zuppan C, Weeks DA, Breslow N, et al. Focal versus diffuse anaplasia in Wilms tumor–new definitions with prognostic significance: a report from the National Wilms Tumor Study Group. Am J Surg Pathol 1996;20:909–20.
[4] Green DM, Beckwith JB, Breslow NE, Faria P, Moksness J, Finklestein JZ, et al. Treatment of children with stages II to IV anaplastic wilms' tumor: a report from the national wilms' tumor study group. J Clin Oncol 1994;12:2126–31.
[5] Dome JS, Cotton CA, Perlman EJ, Breslow NE, Kalapurakal JA, Ritchey ML, et al. Treatment of anaplastic histology wilms' tumor: results from the fifth national wilms' tumor study. J Clin Oncol 2006;24:2352–8.
[6] D'Angio GJBN, Beckwith JB. The treatment of wilms' tumor: results of the third national wilms' tumor study. Cancer 1989;64:349–60.
[7] Green DM, Breslow NE, Beckwith JB, Finklestein JZ, Grundy PE, Thomas PR, et al. Comparison between single-dose and divided-dose administration of dactinomycin and doxorubicin for patients with Wilms' tumor: a report from the National Wilms' Tumor Study Group. J Clin Oncol 1998;16:237–45.
[8] Kalapurakal JA, Li SM, Breslow NE, Beckwith JB, Macklis R, Thomas PR, et al. Influence of radiation therapy delay on abdominal tumor recurrence in patients with favorable histology Wilms' tumor treated on NWTS-3 and NWTS-4: a report from the National Wilms' Tumor Study Group. Int J Radiat Oncol Biol Phys 2003;57:495–9.
[9] Kalapurakal JA, Dome JS, Perlman EJ, Malogolowkin M, Haase GM, Grundy P, et al. Management of Wilms' tumour: current practice and future goals. Lancet Oncol 2004;5:37–46.
[10] D'Angio GJ, Evans A, Breslow N, Beckwith B, Bishop H, Farewell V, et al. The treatment of wilms' tumor:Results of the second national wilms' tumor study. Cancer 1981;47:2302–11.
[11] Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457.
[12] Peto RP J. Asymptotically efficient rank invariant test procedures. J R Stat Soc Ser A 1972;135:184–206.
[13] Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. Br J Canc 1977;35:1–39.
[14] Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Contr Clin Trials 1996;17:343–6.
[15] Vujanic GM, Harms D, Sandstedt B, Weirich A, de KJ, Delemarre JF. New definitions of focal and diffuse anaplasia in Wilms tumor: the International Society of Paediatric Oncology (SIOP) experience. Med Pediatr Oncol 1999;32:317–23.
[16] de Kraker J, Graf N, van TH, Pein F, Sandstedt B, Godzinski J, et al. Reduction of postoperative chemotherapy in children with stage I intermediate-risk and anaplastic Wilms' tumour (SIOP 93-01 trial): a randomised controlled trial. Lancet 2004;364:1229–35.
[17] Bardeesy N, Beckwith JB, Pelletier J. Clonal expansion and attenuated apoptosis in Wilms' tumors are associated with p53 gene mutations. Cancer Res 1995;55:215–9.
[18] Bardeesy N, Falkoff D, Petruzzi MJ, Nowak N, Zabel B, Adam M, et al. Anaplastic Wilms' tumour, a subtype displaying poor prognosis, harbours p53 gene mutations. Nat Genet 1994;7:91–7.
[19] Hill DA, Shear TD, Liu T, Billups CA, Singh PK, Dome JS. Clinical and biologic significance of nuclear unrest in Wilms tumor. Cancer 2003;97:2318–26.
[20] Maschietto M, Williams RD, Chagtai T, Popov SD, Sebire NJ, Vujanic G, et al. TP53 mutational status is a potential marker for risk stratification in Wilms tumour with diffuse anaplasia. PLoS One 2014;9:e109924.
[21] Ooms AH, Gadd S, Gerhard DS, Smith MA, Guidry Auvil JM, Meerzaman D, et al. Significance of TP53 mutation in wilms tumors with diffuse anaplasia: a report from the children's Oncology group. Clin Cancer Res 2016;22:5582–91.