Clinical Trials and Epidemiology

Reflections of the Statistician for the

National Wilms Tumor Study

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06 December 2012
Wilms Tumor (WT) or nephroblastoma

- Embryonal tumor of kidney diagnosed in childhood
  - Modal age 2-4 years
  - Triphasic histology
    - Mixture of stromal, blastemal, epithelial cells

- Exceedingly rare, yet 6% of childhood tumors
  - Incidence 1:10,000 before age 15 yrs in West

- Major success story for modern chemotherapy
  - 90% died in 1900
  - 90% lived in 2000

- Model for study of cancer treatment and etiology
Part III. Clinical Significance

CLINICAL STUDIES OF ACTINOMYCIN D WITH SPECIAL REFERENCE TO WILMS' TUMOR IN CHILDREN*

Sidney Farber, Giulio D'Angio, Audrey Evans, Anna Mitus
Children's Cancer Research Foundation, Children's Medical Center, and Harvard Medical School, Boston, Mass.

This publication commemorates the 20th anniversary of the discovery of actinomycins by Selman A. Waksman. The vast importance of products of the actinomycyes in the treatment of infectious disease is now a part of medical history. Twelve years after the isolation of actinomycin by Waksman and Woodruff in 1940,¹ Hackmann² in 1952 demonstrated the carcinolytic effect of actinomycin C. Studies made by Ravina and others in 1954³ pointed clearly to the possible usefulness of this substance in the treatment of some forms of cancer in man, such as Hodgkin's disease. The studies summarized in this paper had their origin in discussions with Waksman in 1954. The antibiotic selected for initial study, actinomycin D, very quickly proved to be, on the basis of weight, the most powerful anticancer agent against transplanted tumors in the mouse that we had studied up to that time.⁴ ⁵ Clinical studies, begun as soon as extensive toxicological studies were completed, demonstrated no value in the treatment of acute leukemia in children. When administered to

NWTSG circa 1984
NWTS Patient Entries: 9,425 during 1969-2002
Irradiation of Renal Fossa, by Study

Chemotherapy Regimens, by Study

Ten Year Survival by Study

![Graph showing ten year survival rates by study, with different lines for NWTS-1, NWTS-2, NWTS-3, NWTS-4, and NWTS-5. The x-axis represents time in years since Wilms tumor diagnosis, and the y-axis represents percent surviving.](image)
Histology of Wilms Tumor

Ten Year Survival by Histologic Type

![Graph showing survival rates by histologic type.](image-url)
National Wilms Tumor Late Effects Study
NIH Grant R01 CA54498 (1991-2017)

- Principal Investigator & Study Statistician
  ▶ Norman Breslow, PhD

- Co-Investigator & Study Chair
  ▶ Daniel Green, MD

- Project Manager
  ▶ Patricia Norkool, MA

- Database Manager
  ▶ Susan Peterson, MBA

- Chief Data Coordinator
  ▶ Janice Takashima, BA
NWTS Late Effects Study: Specific Aims

• Study mortality
  ▶ in comparison with national population rates (SMR)
    ○ record match with National Death Index (NDI)
  ▶ by cause of death, decade of and time since diagnosis

• Determine incidence of targeted endpoints
  ▶ Congestive heart failure (CHF)
  ▶ Secondary malignant neoplasms (SMN)
  ▶ End stage renal disease (ESRD)
    ○ record match with US Renal Data System (USRDS)
NWTS Late Effects Study: Specific Aims

- Study reproductive risks after WT, radiation, chemo
  - pregnancy complications
  - low birth weight
  - congenital malformations in offspring

- Contribute to genetic epidemiology of WT
  - WT in offspring (recurrence risk)
  - Descriptive studies of familial WT, age-at-onset, birth weights, precursor lesions, clinical and path data, etc.
  - Collaborative studies with molecular biologists
    - case finding resource
SMR for all cause mortality: NWTS 1-4

Cotton CA et al., J Clin Oncol;27:1304-1309, 2009
# International Collaborative Study: SMN & WT

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N. America</th>
<th>Britain</th>
<th>Nordic</th>
<th>Combined</th>
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<tbody>
<tr>
<td>No. WT pts.</td>
<td>8,884</td>
<td>2,893</td>
<td>1,574</td>
<td>13,351</td>
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<tr>
<td>Age (mean yr)</td>
<td>3.7 ± 2.6</td>
<td>3.5 ± 2.6</td>
<td>3.6 ± 2.7</td>
<td>3.6 ± 2.6</td>
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<tr>
<td>FU (med yr)</td>
<td>12.1</td>
<td>9.2</td>
<td>10.7</td>
<td>11.6</td>
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<tr>
<td>SIR solid tumors</td>
<td>104/20.8=5.0</td>
<td>41/8.2=5.0</td>
<td>29/5.0=5.8</td>
<td>174/34.0=5.1</td>
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<tr>
<td>SIR leukemias</td>
<td>24/3.9=6.2</td>
<td>4/1.1=3.5</td>
<td>0/0.7=0.0</td>
<td>28/9.7=2.9</td>
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<tr>
<td>Cum. risk solid tumor ages 15-40</td>
<td>5.8 ± 1.0 %</td>
<td>6.6 ± 1.3 %</td>
<td>7.5 ± 2.0 %</td>
<td>6.7 ± 0.8 %</td>
</tr>
</tbody>
</table>

Breslow NE et al., *Int J Cancer* **127**:657-666, 2010
International Collaborative Study: SMN & WT
Breast Cancer in Female WT Survivors

- Study population: 2,488 female survivors from US & Canada
  - Diagnosed before and survived to age 15 years
  - Followed through December, 2010

- Binary exposure indicator: any chest radiation therapy (RT)
  - 87% “exposed” had single dose to entire lung region
    - Ignore boosts to partial lung regions
  - Doses closely concentrated about protocol specifications
    - 14 Gy for NWTS 1-2
    - 12 Gy for NWTS 3-4

Lange, Takashima, Peterson, Green, Breslow *submitted*, 2012
Breast Cancer in Female WT Survivors

![Graph showing probabilities of breast cancer in female WT survivors by age and chest radiation treatment status.]
# Breast Cancer in Female WT Survivors

<table>
<thead>
<tr>
<th></th>
<th>No. of subjects</th>
<th>No. Breast Ca.</th>
<th>p-value</th>
<th>Cum Inc Age 40</th>
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<tr>
<td></td>
<td></td>
<td>Obs.</td>
<td>Exp.</td>
<td>SIR</td>
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<tr>
<td><strong>Total</strong></td>
<td>2,288</td>
<td>23</td>
<td>2.4</td>
<td>9.8</td>
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<tr>
<td>Chest RT (any)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2,117</td>
<td>10</td>
<td>1.9</td>
<td>5.2</td>
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<tr>
<td>Yes</td>
<td>371</td>
<td>13</td>
<td>0.4</td>
<td>29.7</td>
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<tr>
<td>Age at WT diagnosis (yrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0-9</td>
<td>2,396</td>
<td>16</td>
<td>2.1</td>
<td>7.5</td>
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<tr>
<td>10-14</td>
<td>92</td>
<td>7</td>
<td>0.2</td>
<td>32.4</td>
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*Adjusted for Chest RT*
<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Risk</th>
<th>95% CI</th>
<th>p-value</th>
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<tr>
<td>Gender (F vs M)</td>
<td>4.5</td>
<td>(1.6, 12.6)</td>
<td>0.004</td>
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<tr>
<td>DOX (100mg/M²)</td>
<td>3.2</td>
<td>(1.8, 5.7)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Lung RT (10 Gy)</td>
<td>1.6</td>
<td>(1.0, 2.5)</td>
<td>0.062</td>
</tr>
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<td>L Fossa RT (10 Gy)</td>
<td>1.8</td>
<td>(1.2, 2.8)</td>
<td>0.010</td>
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<tr>
<td>R Fossa RT (10 Gy)</td>
<td>1.0</td>
<td>(0.7, 1.3)</td>
<td>0.770</td>
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Congestive Heart Failure: Pts Treated w/ DOX
Knudson’s 2-Hit Model

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 4, pp. 820–823, April 1971

Mutation and Cancer:  Statistical Study of Retinoblastoma

ALFRED G. KNUDSON, JR.

Graduate School of Biomedical Sciences and M. D. Anderson Hospital and Tumor Institute,
The University of Texas at Houston, Houston, Texas 77025

Communicated by James V. Neel, February 8, 1971

ABSTRACT  Based upon observations on 48 cases of
retinoblastoma and published reports, the hypothesis is
developed that retinoblastoma is a cancer caused by two
mutational events. In the dominantly inherited form, one
mutation is inherited via the germinal cells and the second
occurs in somatic cells. In the nonhereditary form, both
mutations occur in somatic cells.

The second mutation produces an average of three
retinoblastomas per individual inheriting the first muta-
tion. Using Poisson statistics, one can calculate that this
number (three) can explain the occasional gene carrier
who gets no tumor, those who develop only unilateral
tumors, and those who develop bilateral tumors, as well
as explaining instances of multiple tumors in one eye.

This value for the mean number of tumors occurring in
genic carriers may be used to estimate the mutation
rate for each mutation. The germinal and somatic rates for
the first, and the somatic rate for the second, mutation,
are approximately equal. The germinal mutation may arise
in some instances from a delayed mutation.
Knudson’s 2-Hit Model

Knudson Model:
Retinoblastoma
- Bilateral disease (□)
  ▶ Younger ages at Dx
  ▶ 1-hit (exponential) distribution
- Unilateral disease (○)
  ▶ Older ages at Dx
  ▶ 2-hit distribution
Knudson-Comings 2-Hit Model

“Mutation and Cancer:  
A Model for Wilms’ Tumor of the Kidney”


- Hereditary cases = 38% of total (later revised to 15%)
  - 1-hit (exponential) age distribution, median 2 years
  - Bilaterals, familials, WAGR (aniridia syndrome) cases

- Sporadic cases
  - 2-hit age distribution, median 3-4 years
  - Unilaterals, hemihypertrophy and GU anomaly cases

- Lack of familial cases (<1%) ascribed to poor survivorship or impaired reproductive capacity

- Nephroblastomatosis proposed as precursor lesion
LOH at 11p13 as Knudson’s Second Hit

- Mutation in $WT1$ at 11p13 on paternal chromosome
- Mutation inherited by daughter
- Loss-of-heterozygosity (LOH) in both tumors of daughter
  - Somatic recombination (R)
  - Chromosome loss (L)
- But $WT1$ mutations rare
  - < 5 % germline
  - 10-15% somatic

Implications of the 2-Hit Model

• More bilateral tumors than unilateral, multifocal
  ▶ IID Poisson distributions for ≠ tumors in each kidney

• Similar ages-at-onset for hereditary cases: familial, bilateral
  and unilateral, multifocal disease
  ▶ Originally, 1-hit (exponential) distribution
  ▶ Later modified: “declining population of susceptible cells”

• Heritability (\(H\)) estimable from rates of bilaterality (\(B\)) in
  patients with familial vs. sporadic disease (Pr(\(H\mid B\)) = 1)

\[
\Pr(H) = \frac{\Pr(HB)}{\Pr(B\mid H)} = \frac{\Pr(H\mid B)\Pr(B)}{\Pr(B\mid H)} = \frac{\Pr(B)}{\Pr(B\mid H)}
\]
Epidemiological Features of Wilms' Tumor: Results of the National Wilms' Tumor Study\textsuperscript{1,2}

Norman E. Breslow\textsuperscript{3,4} and J. Bruce Beckwith\textsuperscript{5,6}

ABSTRACT—Nearly 2,000 children with Wilms' tumor registered in a national clinical trial during 1969–81 showed high rates of aniridia, hemihypertrophy, cryptorchidism, hypospadias, and other genitourinary anomalies. Patients with bilateral disease, who constituted 5% of the total, had younger ages at diagnosis and an increased incidence of congenital anomalies and renal blastemal rests. Those with multicentric unilateral lesions had more blastemal rests but were otherwise indistinguishable from the unicentric cases. The 20 familial cases had none of the features usually associated with genetic tumors: neither younger ages nor an increase in bilaterality nor associated congenital anomalies. These observations suggest that the fraction of Wilms’ tumors that is due to an inherited mutation may be substantially smaller than previously supposed and support the concept that the disease arises from a variety of pathogenetic pathways.—JNCI 1982; 68:429–436.

Several features of the epidemiology of Wilms' tumor suggest that genetic factors may play a major role in its etiology. The occurrence of the disease is remarkably con-
NWTS Observations Conflict w/ 2-Hit Model

- More unilateral, multifocal than bilateral cases; ages-at-onset intermediate between unilateral, unifocal and bilateral

- $\Pr(H)$ estimated as $\frac{656}{9,425}/\frac{20}{138} = 45\%$ (latest data), yet
  - Low prevalence of familial WT ($\sim 1.4\%$)
  - Low recurrence rate in offspring ($< 3\%$)

- Discovery of two distinct precursor lesions (ILNR & PLNR)
  - Distinct age-at-onset distributions
  - Associated with different congenital anomalies

“WT ... seems to represent more than one genetic entity.”

Genomic Imprinting and Wilms Tumor

- Expression depends on parental origin
  - Allele inherited from Mother switched off (imprinted)
  - Allele inherited from Father expressed
- Loss of imprinting (LOI)
  - Both alleles expressed
- LOI of IGF2 @ 11p15 observed in ~70% WT
- Other genes *paternally* imprinted
  - *e.g.*, H19 @ 11p15
Genetic and Epigenetic Evidence for Heterogeneity

- Multiple familial WT genes
  - $WT1 \oplus 11p13$, $FWT1 \oplus 17q$, $FWT2 \oplus 19q$, ...

- Mutations in $WT1$, $CTNNB1 \oplus 3p22$ and $WTX \oplus Xq11$
  - Any 1, 2 ($WT1\oplus$) or all 3 may be mutated
  - Collectively found in $\sim 1/3$ of WT

- Loss-of-imprinting (LOI) of $IGF2$ in $\sim 70\%$ of WT
Wilms Tumor - Aniridia (WAGR) Syndrome

Wilms tumor
Aniridia (lack of iris)
GU anomalies (males)

- hypospadias (abnormal opening in penis)
- cryptorchism (undescended testis)

Retardation (mental)

- Results from constitutional deletion at 11p13
  ▶ deletion of WT1 and PAX6 genes

Denys-Drash Syndrome (DDS)

1. Distinct congenital nephropathy (diffuse mesangial sclerosis) that often leads to end stage renal disease (ESRD)

2. Wilms tumor

3. Disorders of sexual differentiation (“ambiguous genitalia”)

Caused by constitutional point mutations in \( WT1 \)

Denys P et al., Archives Françaises de Pédiatrie 24:729-39, 1967
Drash A et al., Journal of Pediatrics 76:585-93, 1970
NWTS: Prevalence of WAGR and DDS
Cases per 1,000 WT patients

Year of WT diagnosis

Cases per 1,000 WT patients

WAGR
DDS

Nephrogenic Rests as Precursor Lesions

Nephrogenic Rests and Histologic Subtype
Patients with Favorable Histology Wilms Tumor

Percent distribution

- None
- PLNR
- ILNR
- Both

Mixed: n=2,708
Blastema: n=1,851
Epithelial: n=581
Stromal: n=168
Nephrogenic Rests, Gender and Age
Patients with Multifocal Wilms Tumor

... No rests identified; — — ILNR±PLNR; — — PLNR only
Increased Birth Weights of NWTS Patients Suggest a Growth-Factor Excess
Leisenring, Breslow, Evans et al., Cancer Research 54:4680-4683, 1994
Ideal Biological Subtype I

- Deletion or mutation in \( WT1 \) @ 11p13
  - germline (1-hit) or somatic cell (2-hit)

- Intralobar nephrogenic rests (ILNR)
  - Early in embryogenesis from immature nephroblast

- Stromal predominant histology

- Early age-at-onset

- Includes patients with DD/WAGR syndromes, GU anoms

Ideal Biological Subtype II

- Loss of (maternal) imprinting (LOI) of $IGF2$
  - Double dose of growth factor during embryogenesis

- Perilobar nephrogenic rests
  - Arise from mature nephroblasts later in foetus

- Blastemal or epithelial predominant histology

- Later age-at-onset

- Includes patients w/ Beckwith-Wiedemann Syndrome
  - Overgrowth syndrome mapped to 11p15 (locus of $IGF2$)
Epidemiology of Wilms Tumor in Asia

• ~ 1/2 incidence rates of Caucasian children

• Earlier ages-at-onset

• (Slight) male predominance

Epidemiology of Wilms Tumor in Asia

Age at WT Diagnosis in NWTS Asian Americans

Smoothed density * 100

- ILNR
- PLNR only
- Neither

Age at diagnosis (years)
Epidemiology of Wilms Tumor in Asia

- Epidemiology: fewer WT with PLNR precursors
  - 1/56 (2%) in Japanese
  - 7/92 (8%) in Asian Americans (NWTS)
  - 119/5002 (24%) in Caucasian Americans (NWTS)

- Laboratory: LOI @ IGF2 by real time PCR
  - 0/33 (0%) in Japanese WT
  - 13/41 (32%) in Causasian (NZ) WT

- Virtual absence of Type II biological subtype

  Fukuzawa, Breslow, Morrison et al., Lancet, 2004
NWTS/USRDS Study of ESRD

- NWTS 1-4 pts from U.S., complete baseline data
  - Cohort I: 5,526 pts with unilateral WT at Dx
  - Cohort II: 384 pts with bilateral WT (66 metachronous)

- Identified 115 pts w/ ESRD (55 Cohort I, 60 Cohort II)
  - 92 (80%) by NWTS and record linkage to USRDS
  - 13 (11%) by USRDS only (8 LTFU by NWTS)
  - 10 (9%) by NWTS only (3 died of uremia w/o treatment)

- Four subgroups defined by congenital malformations
  - Estimate cumulative incidence of ESRD for each
Cumulative Incidence of ESRD: Unilateral WT

[Graph showing cumulative incidence of ESRD over time since diagnosis of unilateral Wilms tumor.]

- DDS (12/17)
- WAGR (11/37)
- GU (4/125)
- Other (28/5,347)

Time since diagnosis of unilateral Wilms tumor (years)
Laboratory Follow-up

French group motivated by NWTS data to examine adjacent (to tumor, normal) kidney in 7 WAGR patients + controls
WAGR patients had

- Bimodal distribution of glomeruli diameters
- Substantially smaller average glomerulus size
- Suggests specific defect of \( WT1 \) function in ESRD

Dahan, Kamal, Noël et al., *Am J Kid Dis* **49**:793-800, 2007
NWTS Study of Risk Factors for ESRD

- Study cohort: 7,951 patients on NWTS 1-5
  - Excludes pts with DDS, WAGR, GU anomalies
    - 66 cases of ESRD identified among 323 patients
  - Additional exclusions for missing data, non-WT histology

- ESRD in non-WT1 syndromic pts classified in 2 groups
  - ESRD due to progressive bilateral WT ($n=45$)
    - Surgical removal of both kidneys
  - ESRD due to “chronic kidney disease” ($n=55$)
    - Ascribed to various causes

ESRD Risk Factors Study: A priori Hypotheses

• Patients with *metachronous* bilateral disease have higher rates of ESRD due to progressive bilateral WT than those with *synchronous* bilateral disease
  - Bilateral at diagnosis ⇒ renal sparing surgery
  - Wilms tumors that develop while patient is on (or shortly after completion of) anti-tumor therapy are selected to be more aggressive and/or less responsive to treatment

• Clinico-pathologic features associated with “Ideal Biological Subtype I” WT (presumed *WT1* etiology)) increase the risk of ESRD due to chronic renal failure
  - Young age-at-onset
  - Stromal predominant histology
  - Presence of intralobar nephrogenic rests
Cumulative Incidence of ESRD
Due to Progressive Bilateral Wilms Tumor
Cumulative Incidence of ESRD Due to Chronic Kidney Disease
Cumulative Incidence of ESRD Due to Chronic Kidney Disease

- Restricted to patients with unilateral disease
### Results of Multiple Cox Regression
Hazard ratios (HR) for ESRD due to chronic kidney disease

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
<th># Pts</th>
<th>cases</th>
<th>HR</th>
<th>95% CI</th>
<th>p</th>
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<tbody>
<tr>
<td>Age at WT (months)</td>
<td>0-23</td>
<td>1,480</td>
<td>15</td>
<td>2.0</td>
<td>(0.7, 5.9)</td>
<td>0.22</td>
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<tr>
<td></td>
<td>24+</td>
<td>3,999</td>
<td>7</td>
<td>1.0</td>
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<tr>
<td>Histology</td>
<td>Stromal</td>
<td>148</td>
<td>8</td>
<td>7.8</td>
<td>(3.0, 20.6)</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>All others</td>
<td>5,331</td>
<td>14</td>
<td>1.0</td>
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<td></td>
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<tr>
<td>Precursor</td>
<td>None found</td>
<td>3,230</td>
<td>6</td>
<td>1.0</td>
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<tr>
<td>Lesions</td>
<td>ILNR, both</td>
<td>1,116</td>
<td>14</td>
<td>3.8</td>
<td>(1.3, 11.8)</td>
<td>0.02</td>
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<td></td>
<td>PLNR only</td>
<td>1,133</td>
<td>2</td>
<td>0.7</td>
<td>(0.1, 3.7)</td>
<td>0.67</td>
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<tr>
<td>Radiation*</td>
<td>Per group</td>
<td>NA</td>
<td>NA</td>
<td>0.3</td>
<td>(0.1, 1.7)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

* RT to remaining kidney coded 0 = none, 1 = 0.1-14.9, 2 = 15+ Gy

- 5,479 patients from NWTS 3-5 with known rest status
- 22 cases of ESRD (instead of 55 for most univariate analyses)
- Stratified on unilateral vs. bilateral WT
Conclusions from ESRD Risk Factor Study

- *A priori* hypotheses confirmed
  - Patients with metachronous bilateral tumors at higher risk for ESRD due to surgical removal of both kidneys for progressive WT
  - Younger age, stromal histology, intralobar rests predict higher rates of ESRD due to other renal pathologies

- Suggests case-control study of *WT1* mutations among ESRD cases and controls using stored tissue

- **Recommend**: routine surveillance of patients with stromal predominant histology or ILNR
  - Candidates for renal sparing surgery
  - Look closely for signs of renal failure
Lessons from NWTS: Importance of

- Systematic data collection (over decades)
  - Standard definitions and codes (and pathologists)

- Long term follow-up
  - Evaluate consequences of entire treatment policies
  - Identify “late sequelae” (SMN, ESRD) decades after Rx
  - But poor current participation from NWTS-5 survivors, for whom biological material available, threatens future of study

- Close integration of epidemiology with laboratory data
  - Interpret epidemiological results in light of molecular
  - Suggest problems for pathology and molecular study
UW Authors of NWTS Publications

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• Ruth Etzioni
• Wendy Leisenring
• Jane Olson
• Charissa Hogeboom
• Judy Felgenhauer
Thank You, Dan and Audrey!

Married Feb 1, 2005