Clinical Trials and Epidemiology

Reflections of the Statistician for the

National Wilms Tumor Study

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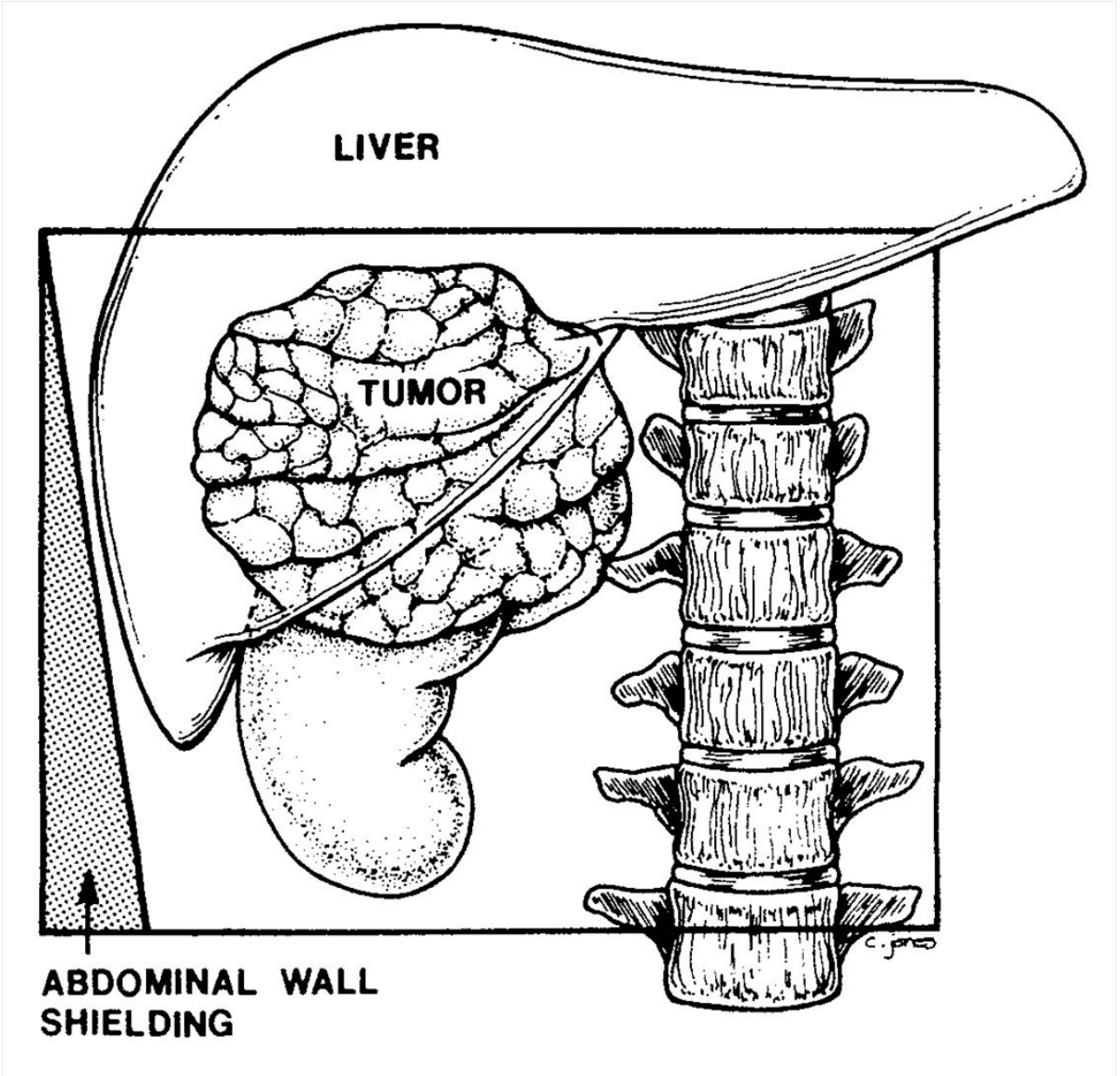
06 December 2012

Schematic of a Wilms Tumor
from the viewpoint of a radiation oncologist



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

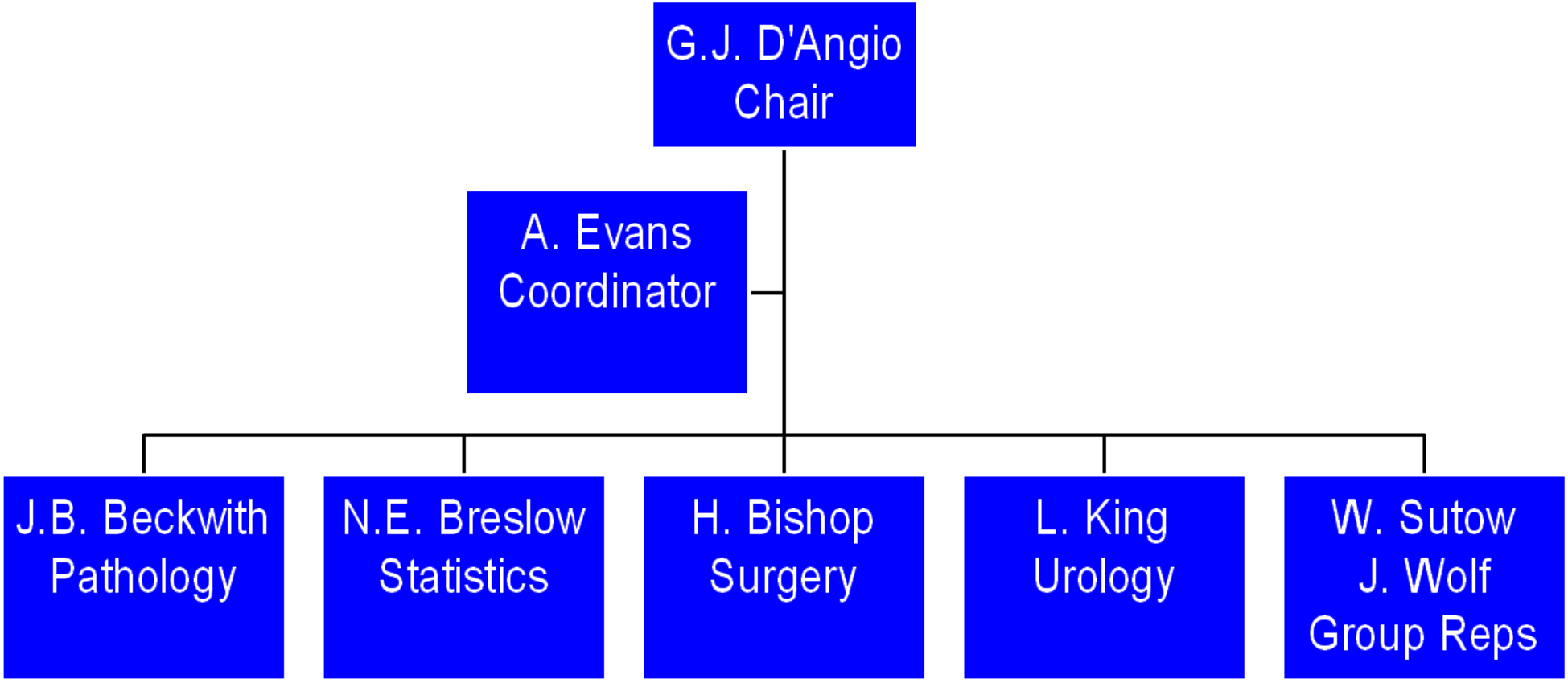
- Exceedingly rare, yet 6% of childhood tumors
  - Incidence 1:10,000 before age 15 yrs in West

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

- Model for study of cancer treatment and etiology

National Wilms Tumor Study (NWTS)

Founding Committee: 1969



Farber, D'Angio, Evans & Mitus: 1960

Annals of the New York Academy of Sciences **89**:421-5, 1960

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

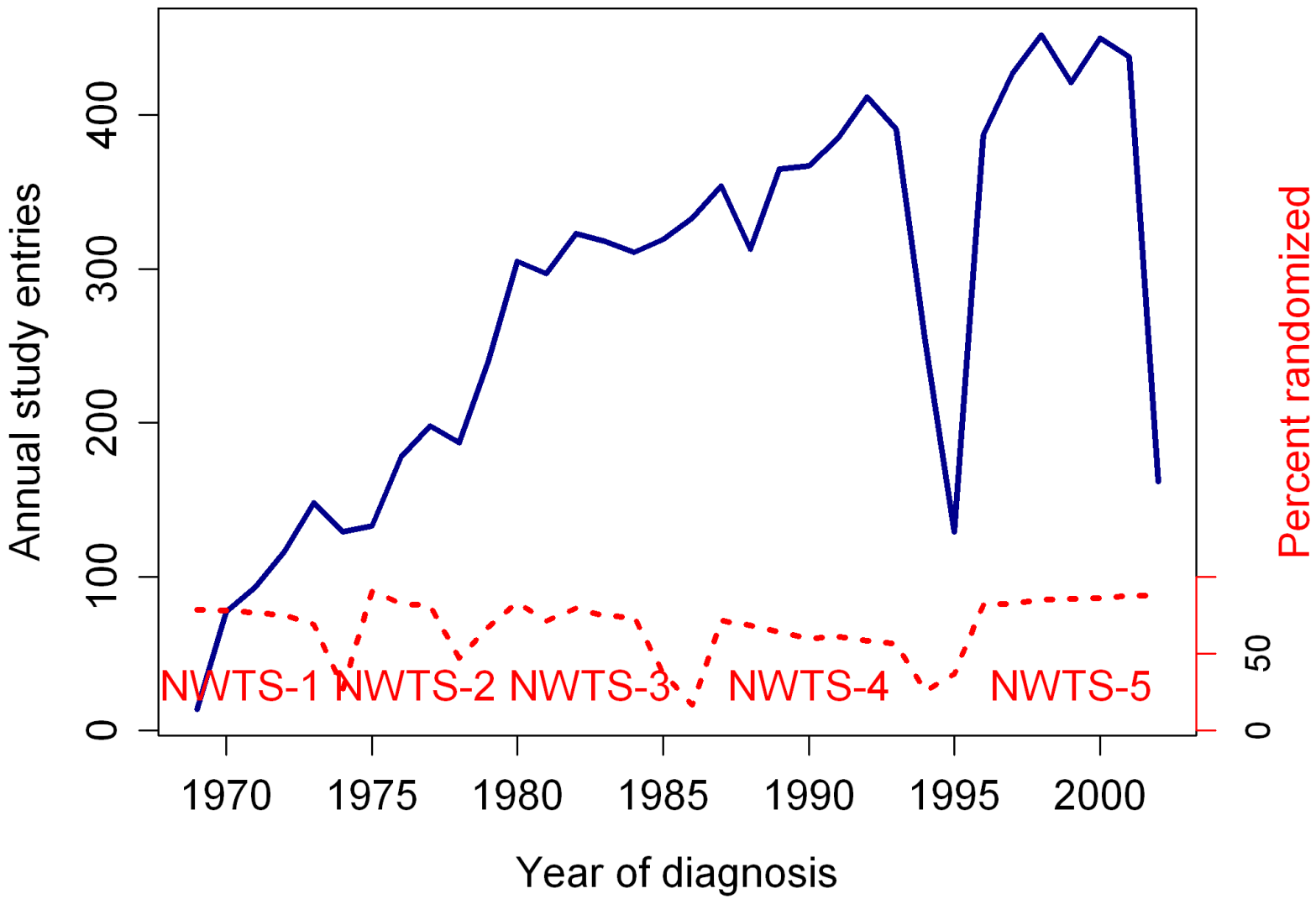
Children's Cancer Research Foundation, Children's Medical Center, and Harvard Medical School, Boston, Mass.

This publication commemorates the 20th anniversary of the discovery of actinomycins by Selman A. Waksman. The vast importance of products of the actinomyces 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] Hackmann[2] in 1952 demonstrated the carcinolytic effect of actinomycin C. Studies made by Ravina and others in 1954[3] 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

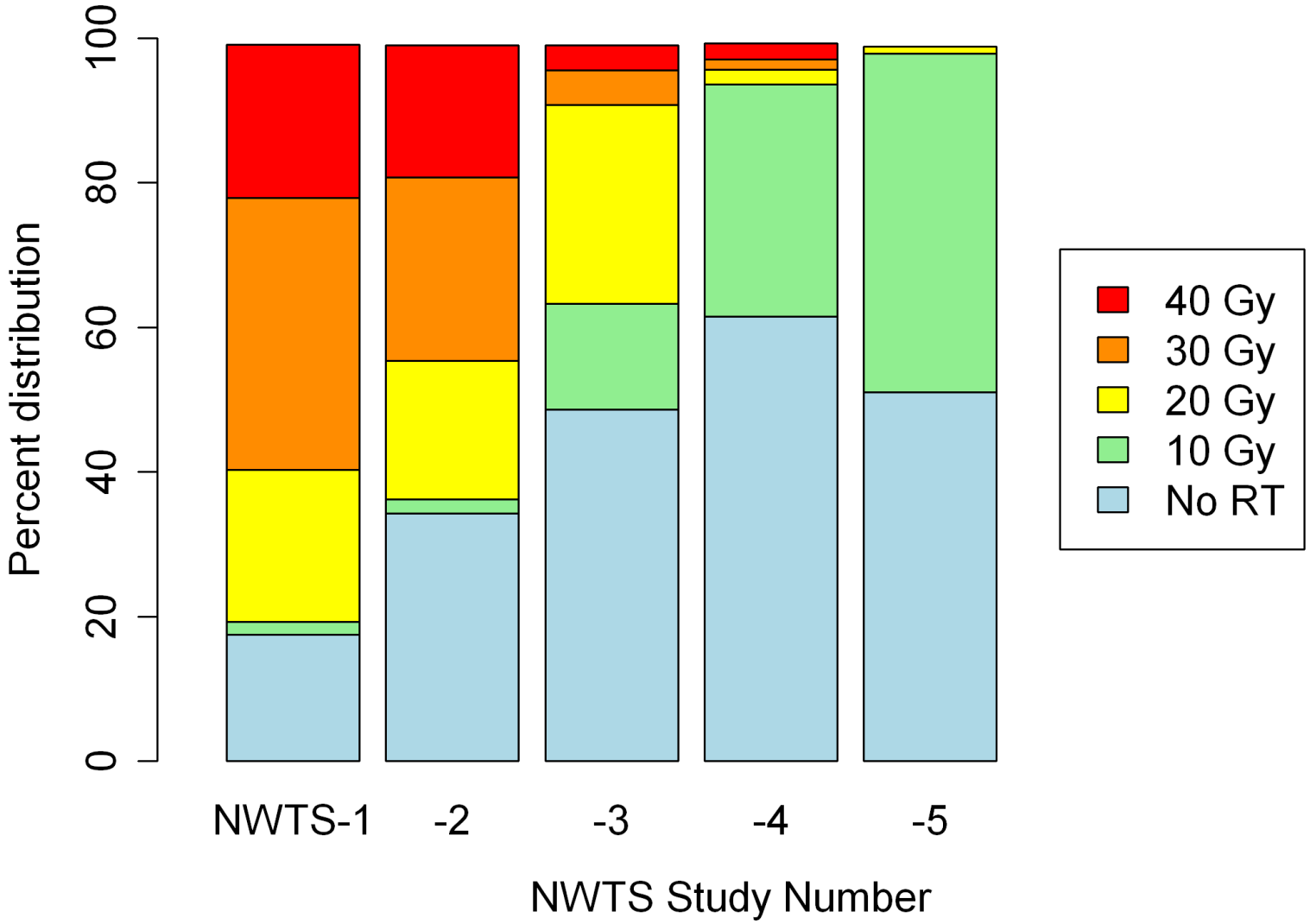
Reprinted in: *J Urol* **168**(6):2560-2562, 2002

NWTSG circa 1984

NWTS Patient Entries: 9,425 during 1969-2002

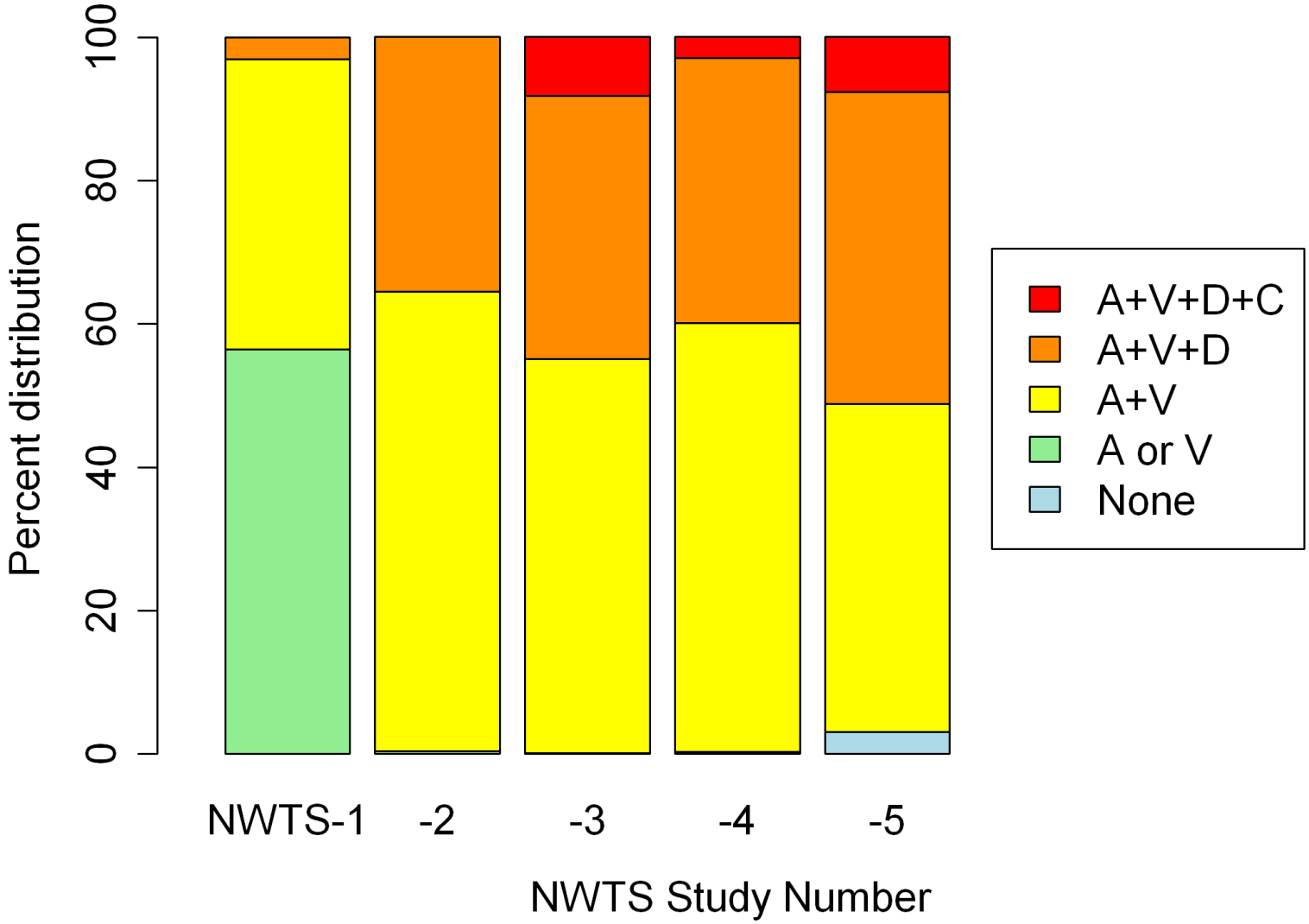


Irradiation of Renal Fossa, by Study



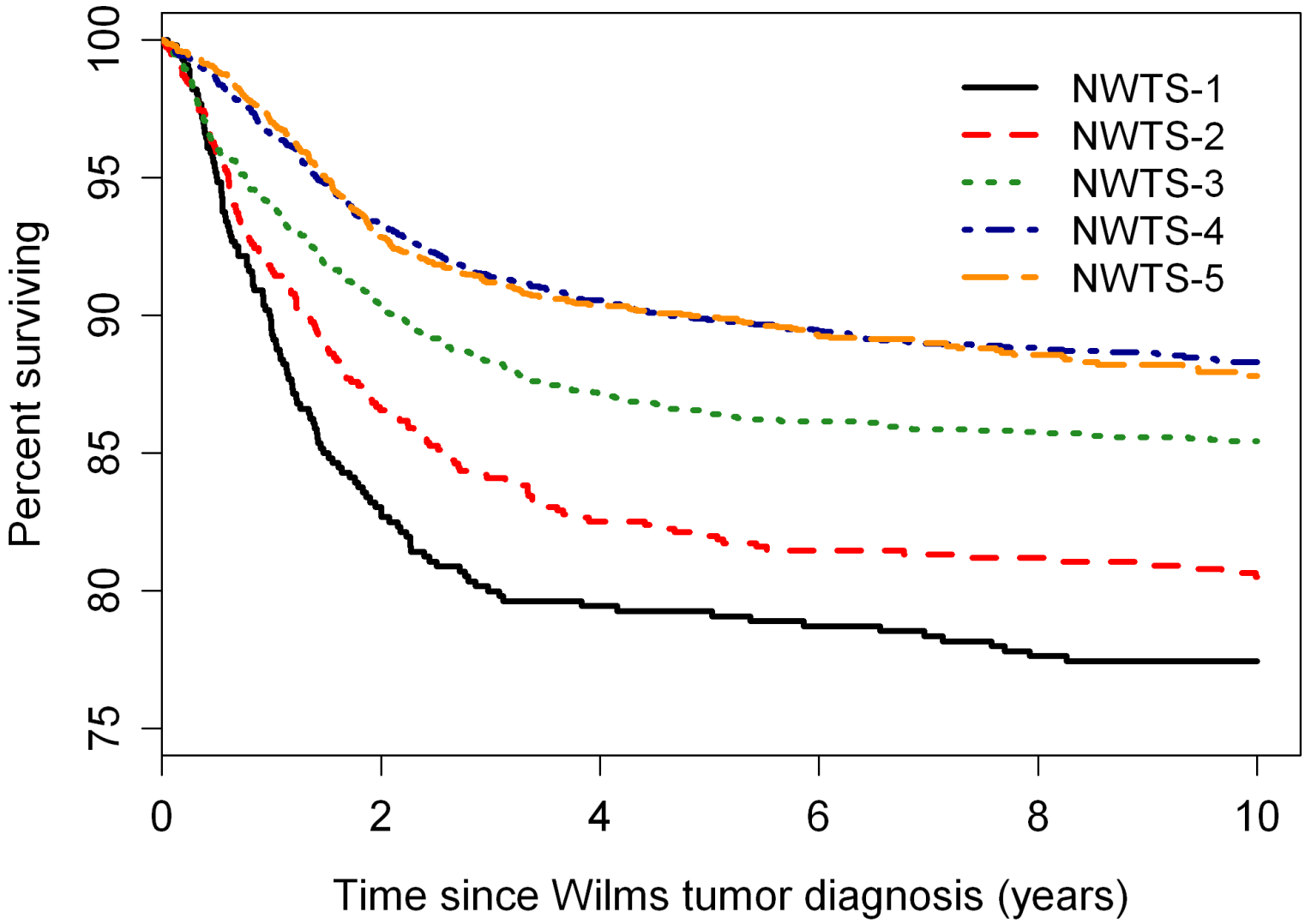
D'Angio GJ *et al.*, *Cancer* **38**:633-646, 1976; **47**:2302-2311, 1981; **64**:349-360, 1989

Chemotherapy Regimens, by Study

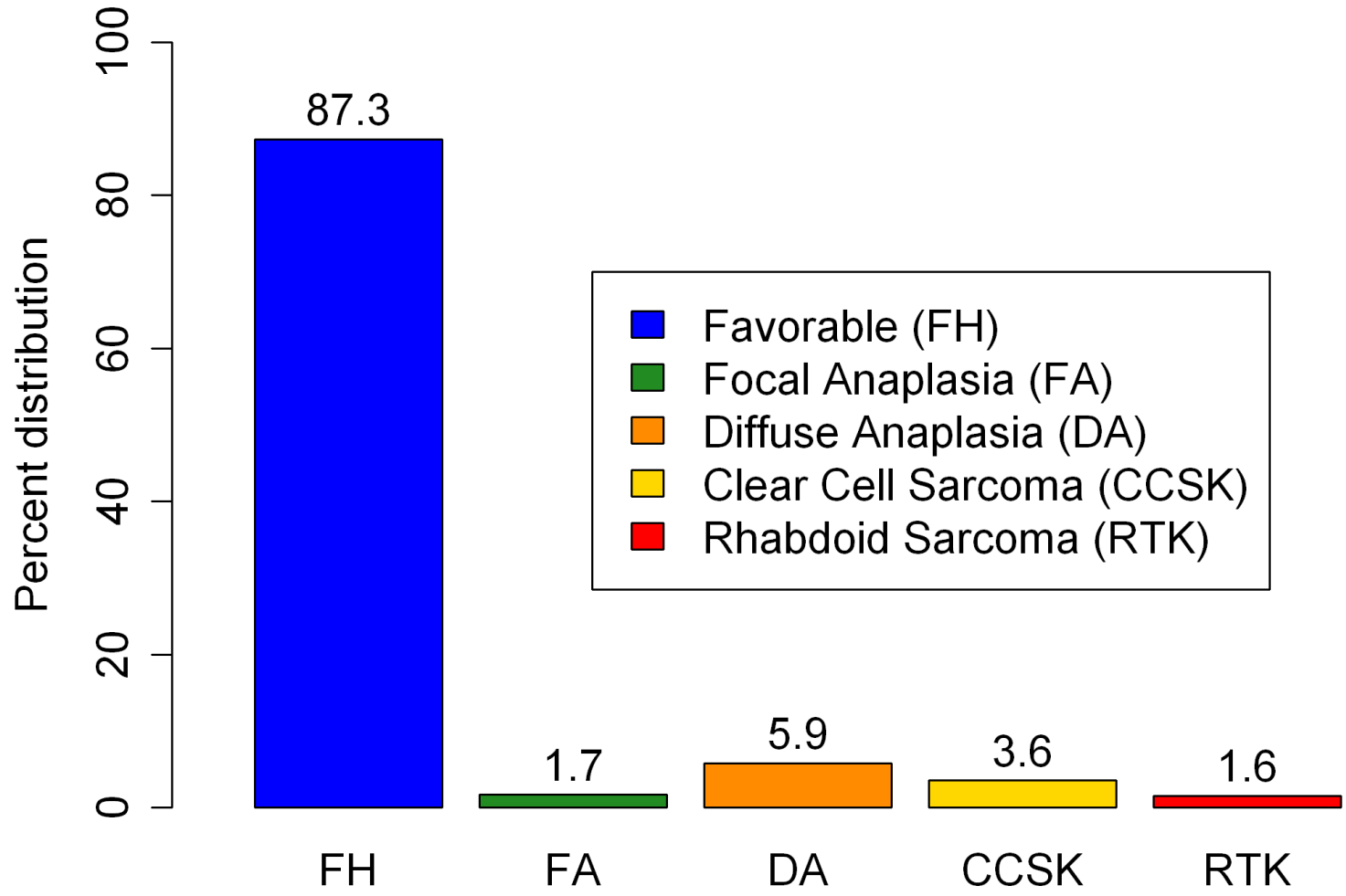


Green DM *et al.*, *J Clin Oncol* **16**:237-245, 1998;
Grundy PE *et al.*, *J Clin Oncol* **23**:7312-7321, 2005

Ten Year Survival by Study

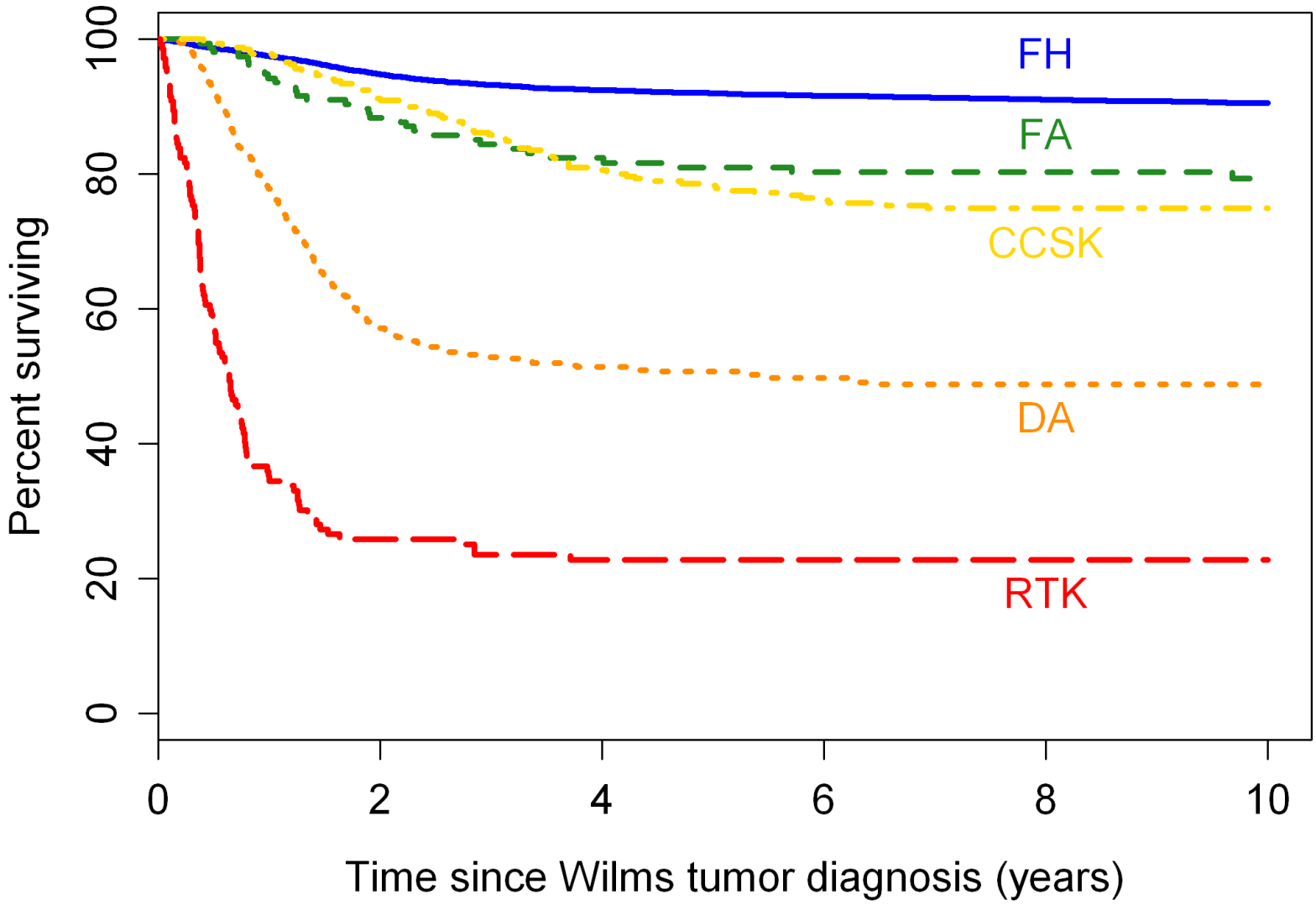


Histology of Wilms Tumor



Beckwith JB, Palmer NF. *Cancer* **41**:1937-1948, 1978

Ten Year Survival by Histologic Type



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

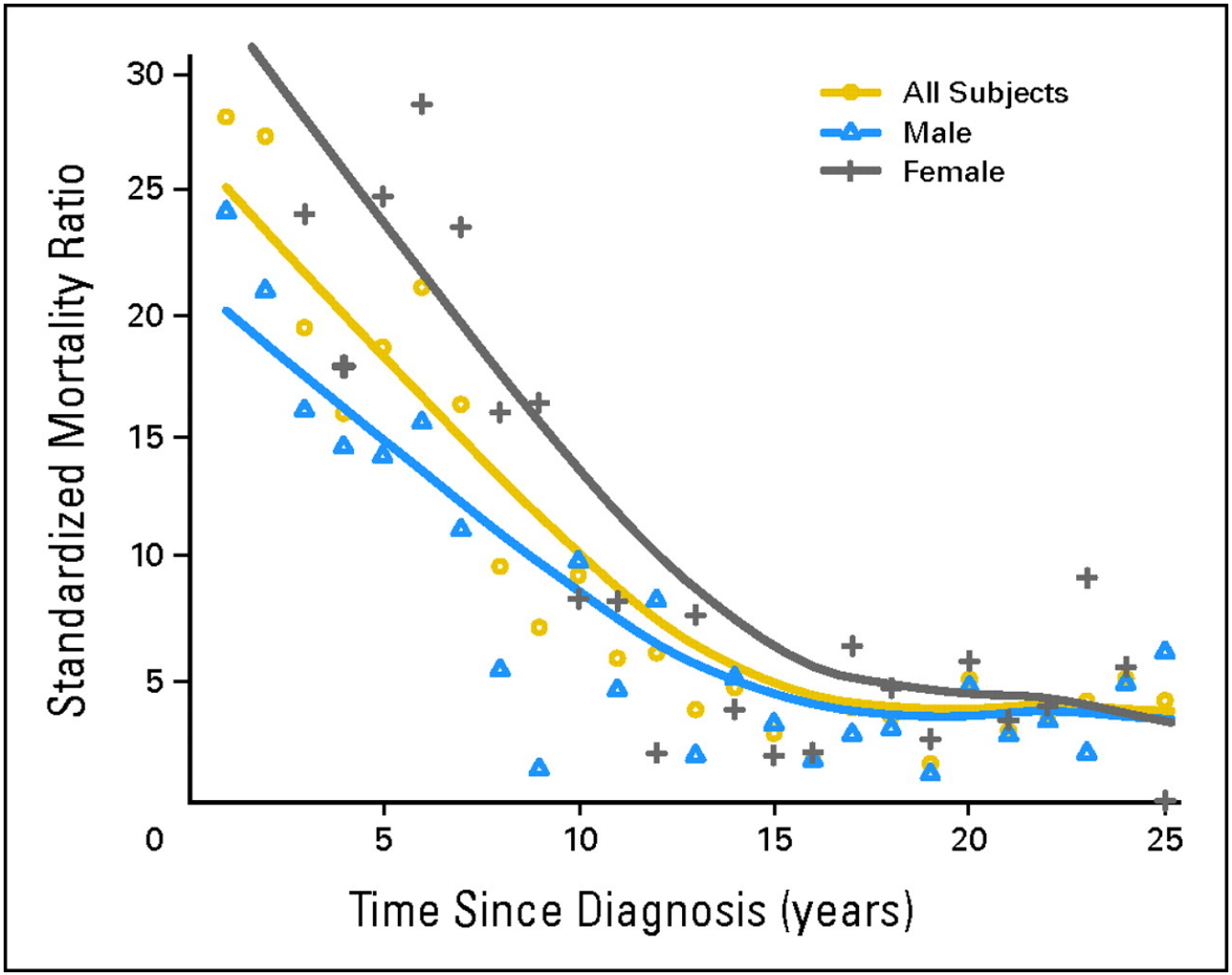
- Study mortality
  - in comparison with national population rates (SMR)
    - record match with National Death Index (NDI)
  - by cause of death, decade of and time since diagnosis

- 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



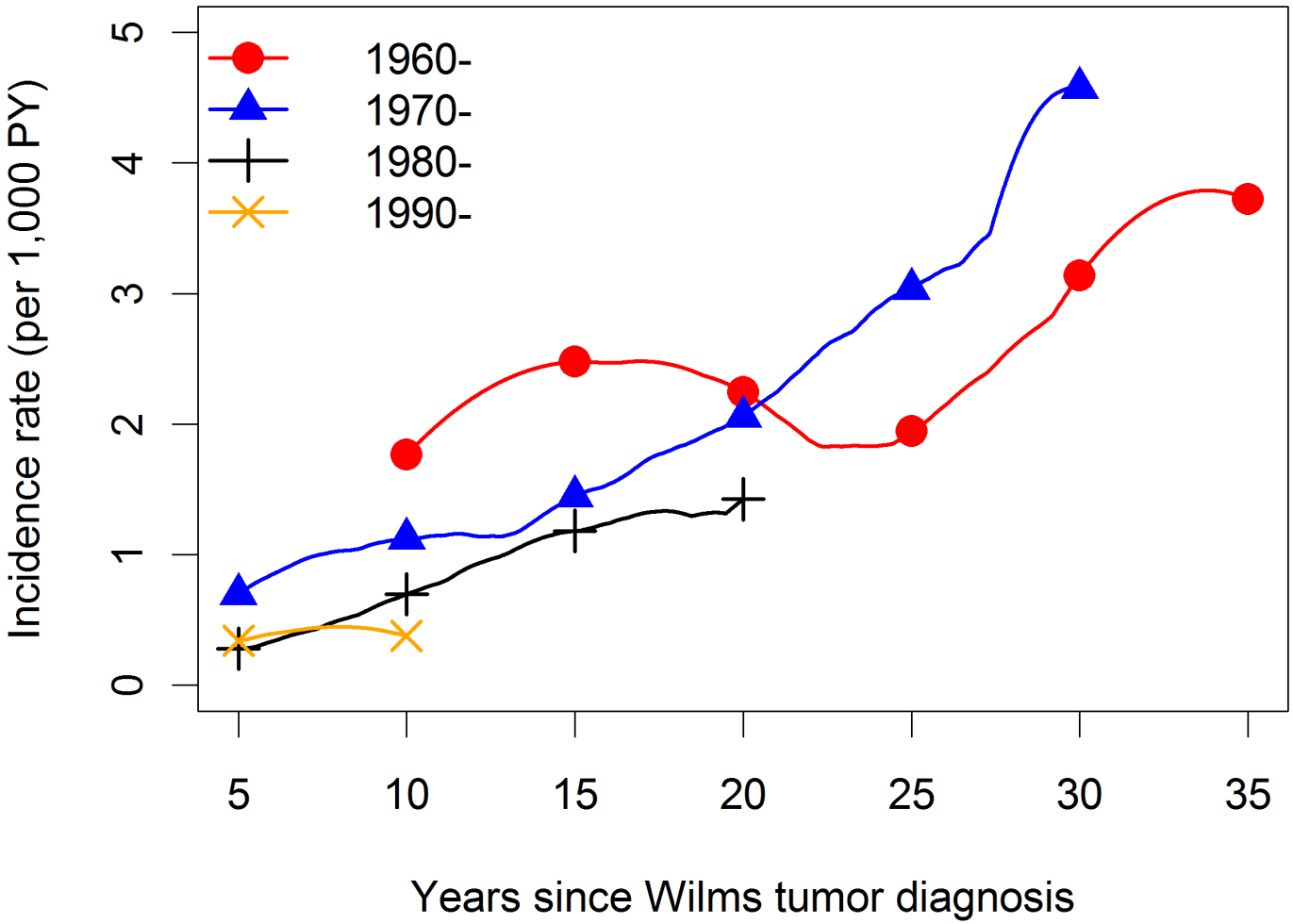
Cotton CA *et al.*, *J Clin Oncol*;**27**:1304-1309, 2009

International Collaborative Study: SMN & WT

Cohort	N. America	Britain	Nordic	Combined
No. WT pts.	8,884	2,893	1,574	13,351
Diagnosis	1969-2002	1962-2002	1960-2004	1960-2004
Age (mean yr)	3.7 ± 2.6	3.5 ± 2.6	3.6 ± 2.7	3.6 ± 2.6
FU (med yr)	12.1	9.2	10.7	11.6
SIR solid tumors	104/20.8=5.0	41/8.2=5.0	29/5.0=5.8	174/34.0=5.1
SIR leukemias	24/3.9=6.2	4/1.1=3.5	0/0.7=0.0	28/9.7=2.9
Cum. risk solid tumor ages 15-40	5.8 ± 1.0 %	6.6 ± 1.3 %	7.5 ± 2.0 %	6.7 ± 0.8 %

Breslow NE *et al.*, *Int J Cancer* **127**:657-666, 2010

International Collaborative Study: SMN & WT



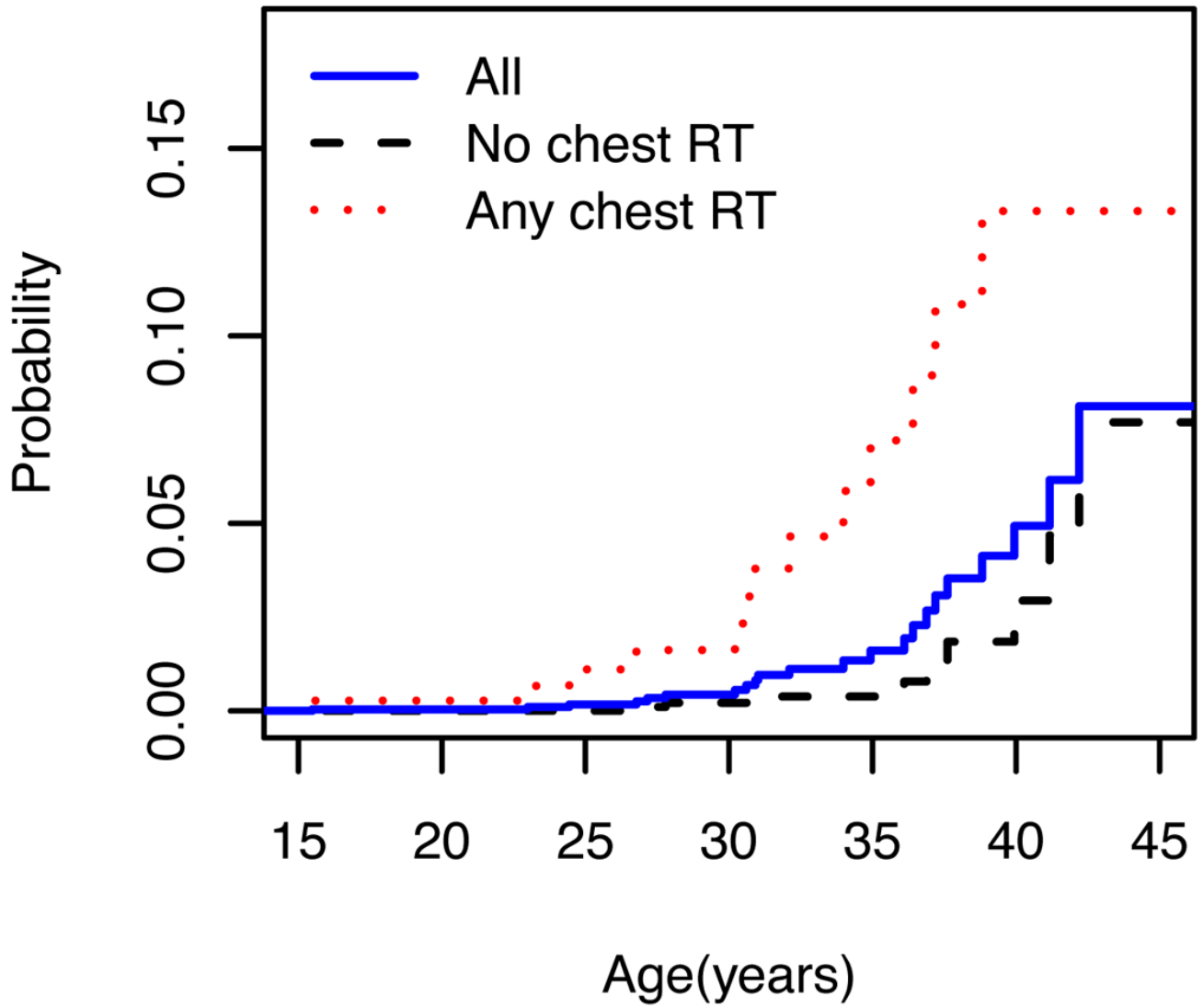
Breast Cancer in Female WT Survivors

- Study population: 2,488 female survivors from US & Canada
  - ▷ Enrolled on NWTS 1-4 (1969-1995)
  - ▷ 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



Breast Cancer in Female WT Survivors

	No. of subjects	No. Breast Ca. Obs.	Exp.	SIR	HR	p-value	Cum Inc Age 40
Total	2,288	23	2.4	9.8	—	—	4.9%
Chest RT (any)							
No	2,117	10	1.9	5.2	—	—	2.9%
Yes	371	13	0.4	29.7	6.5	< 0.001	13.3%
Age at WT diagnosis (yrs)							
0-9	2,396	16	2.1	7.5	—	—	3.6%
10-14	92	7	0.2	32.4	6.0	< 0.001	13.0%

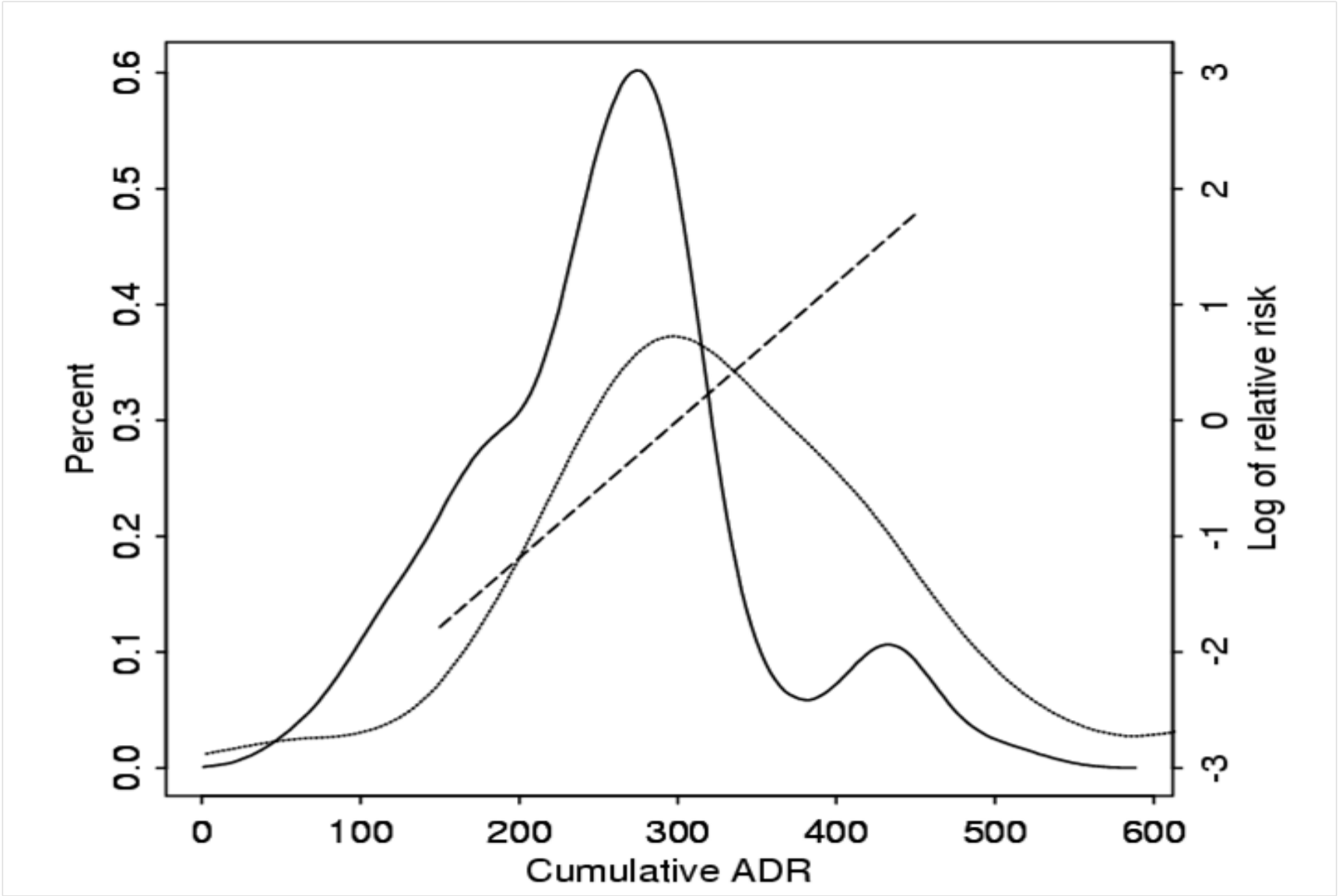
*Adjusted for Chest RT

Congestive Heart Failure: Pts Treated w/ DOX

Variable (unit)	**Relative Risk**	**95% CI**	p-**value**
Gender (F vs M)	4.5	(1.6, 12.6)	0.004
DOX (100mg/M^2)	3.2	(1.8, 5.7)	$<$ 0.001
Lung RT (10 Gy)	1.6	(1.0, 2.5)	0.062
L Fossa RT (10 Gy)	1.8	(1.2, 2.8)	0.010
R Fossa RT (10 Gy)	1.0	(0.7, 1.3)	0.770

Green DM *et al.*, *J Clin Oncol* **19**:1926-1934, 2001

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.

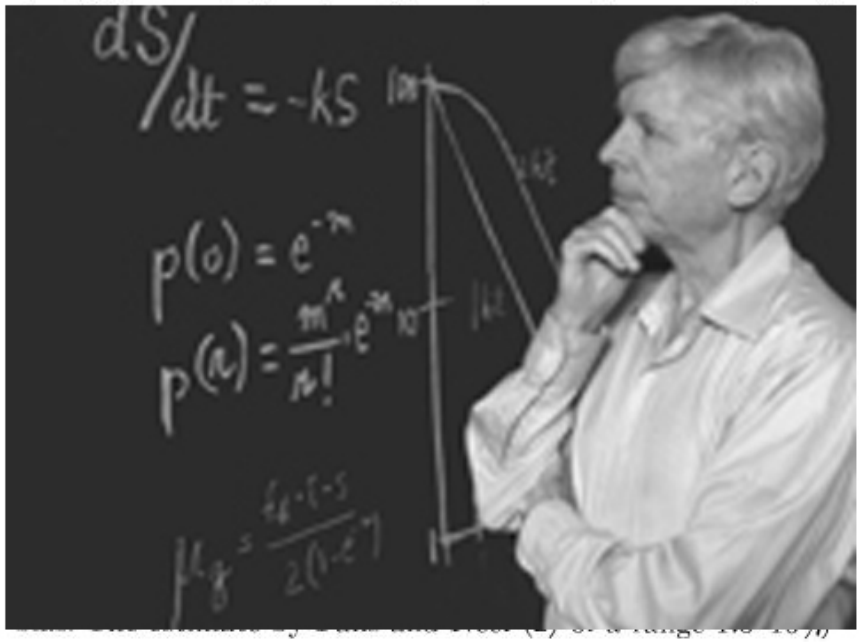
Graduate School of Biomedical Sciences and M. D. Anderson Hospital and Tumor Institute,
The University of Texas at Houston, Houston, Texas 77025

Communicated by James V. Neel, February 8, 1971

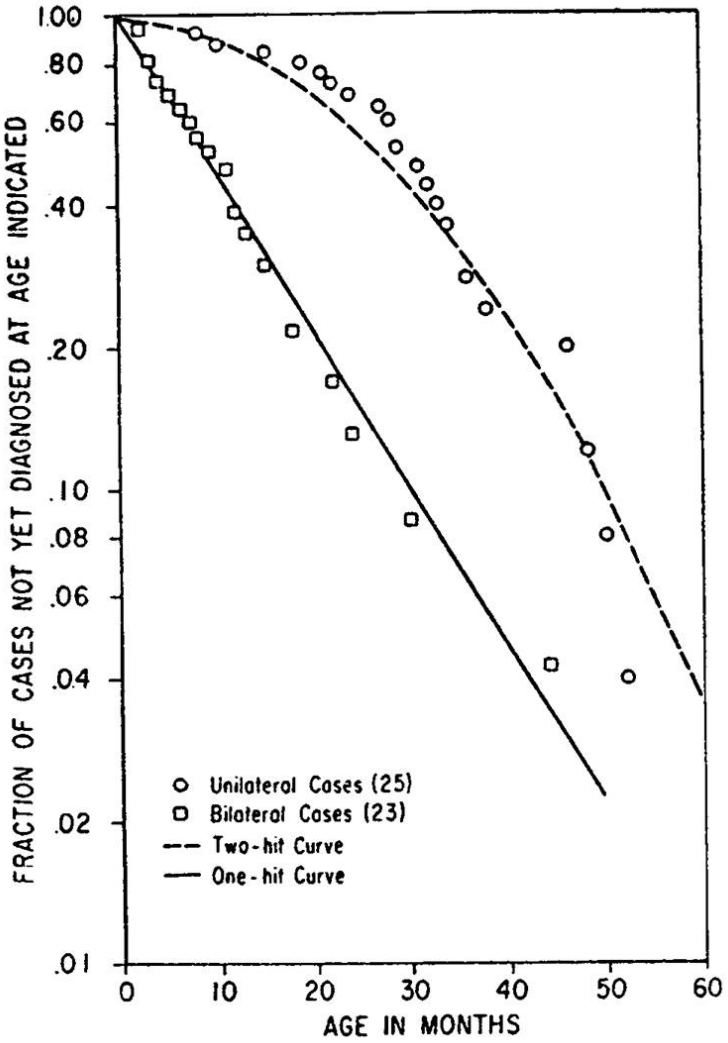
ABSTRACT **Based upon observations on 48 cases of retinoblastoma and published reports, the hypothesis is developed that retinoblastoma is a cancer caused by two mutational events. In the dominantly inherited form, one mutation is inherited via the germinal cells and the second occurs in somatic cells.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

The second mutation produces an average of three retinoblastomas per individual inheriting the first mutation. 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 genetic 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

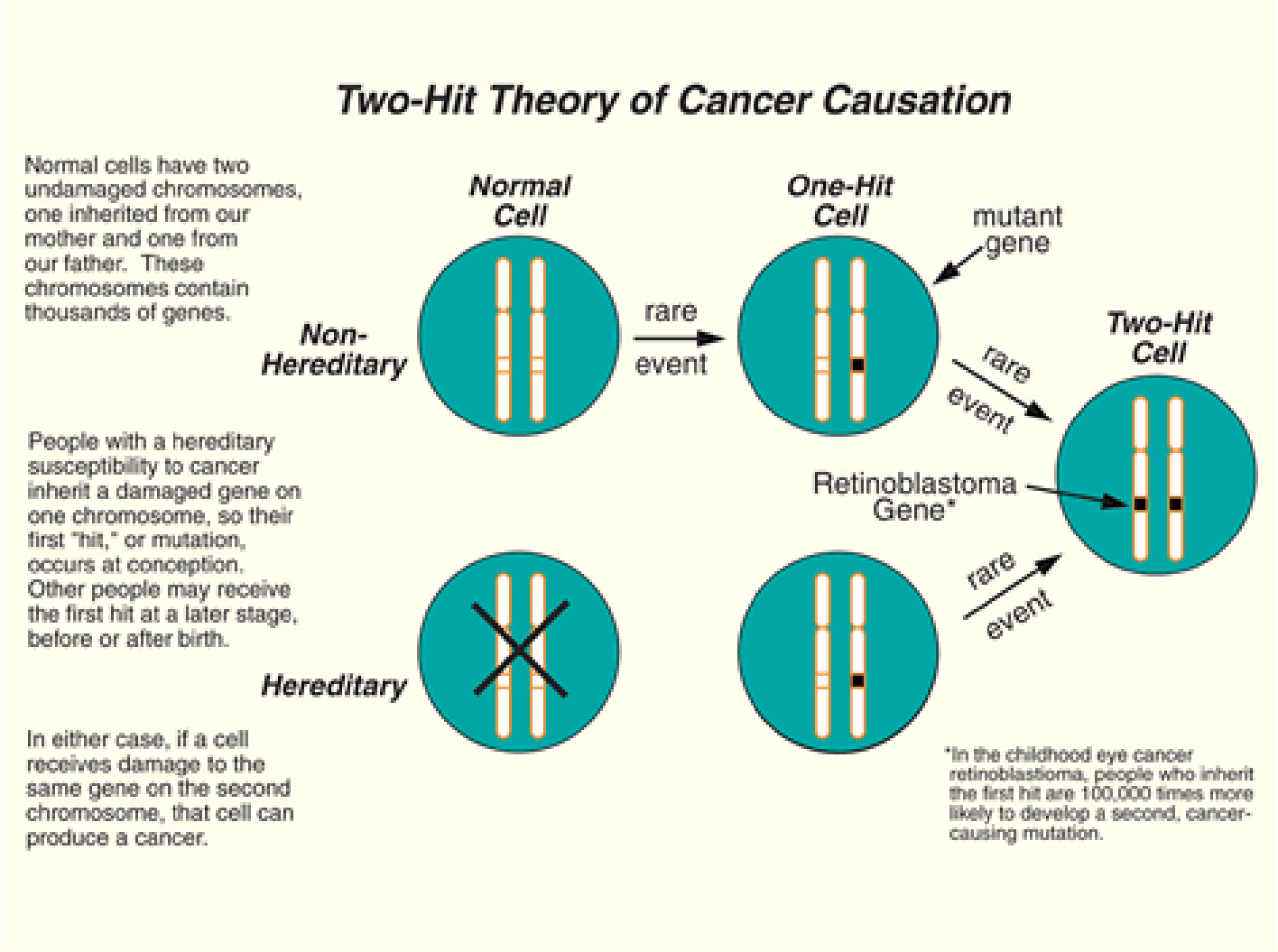


Knudson Model:

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



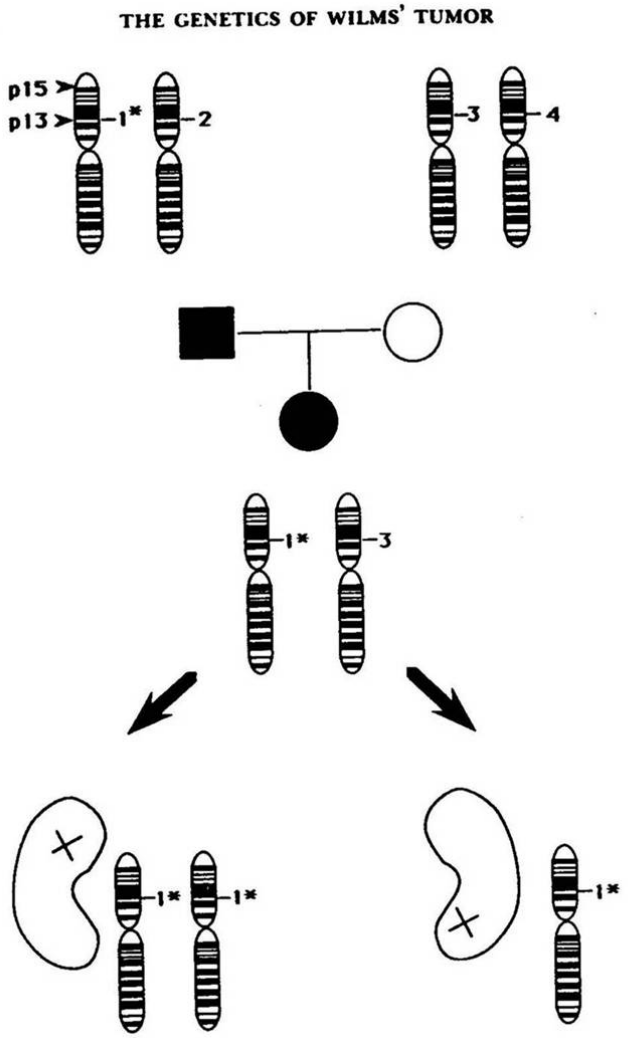
Knudson AG, *PNAS*, 1971; Comings DE, *PNAS*, 1973

"Mutation and Cancer: A Model for Wilms' Tumor of the Kidney"

Knudson AG, Strong LC; *J Nat Cancer Instit* **48**:313-324, 1972

- 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, hemihypertophy 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

Huff, Miwa, Haber *et al.*, *Am J Hum Genetics*, 1991

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 ($\Pr(H|B) = 1$)

$$\Pr(H) = \frac{\Pr(HB)}{\Pr(B|H)} = \frac{\Pr(H|B)\Pr(B)}{\Pr(B|H)} = \frac{\Pr(B)}{\Pr(B|H)}$$

NWTS Observations Conflict w/ 2-Hit Model

Epidemiological Features of Wilms' Tumor: Results of the National Wilms' Tumor Study[1,2]

Norman E. Breslow[3,4] *and* **J. Bruce Beckwith**[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-

patients registered with the study during the past decade, emphasizing particularly the quantitative differences between bilateral–familial versus unilateral–sporadic cases vis-à-vis age at onset, the occurrence of congenital anomalies, and certain histologic findings.

MATERIALS AND METHODS

Study population.—Between October 1969 and March 1981, a completed registration form was submitted to the NWTS Statistical Center for 2,073 patients. Excluded from consideration here were those whose final diagnosis was other than Wilms' tumor: 59 cases of congenital mesoblastic nephroma, 16 neuroblastomas, 7 polycystic kidneys, 6 renal cell carcinomas, and 13 cases with various benign and malignant conditions. Also excluded were 60 patients identified by the NWTS Pathology Center as having monophasic, clear cell, or rhabdoid sarcoma of the kidney (*12*) and 7 patients who were over 15 years of age at the time of diagnosis. This left 1,905 cases for analysis.

NWTS Observations Conflict w/ 2-Hit Model

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

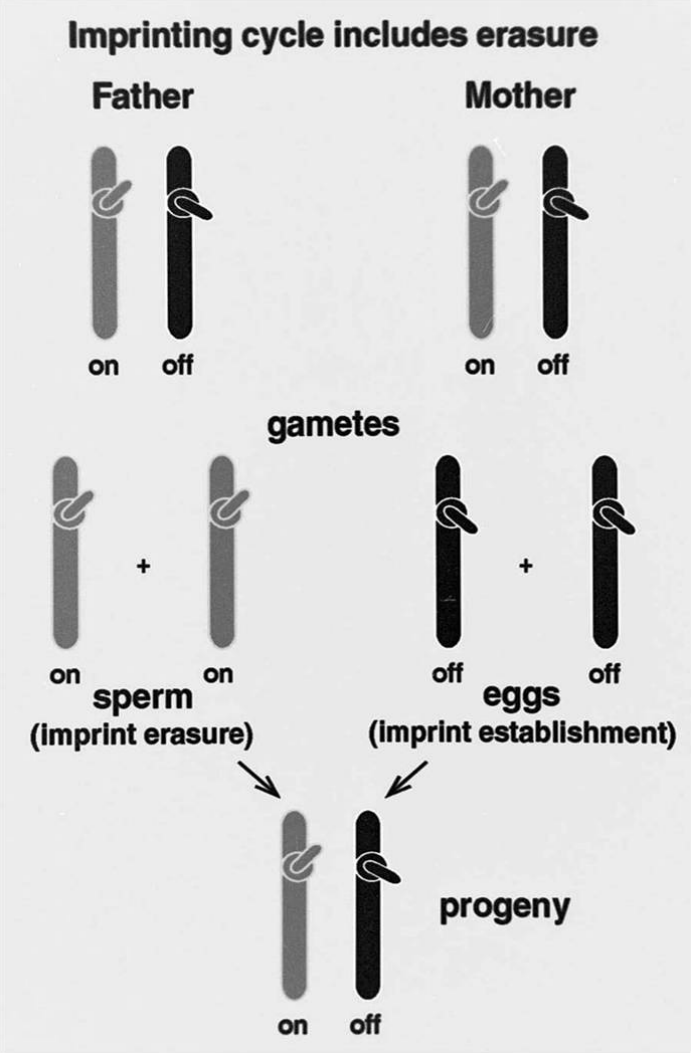
- Pr(H) estimated as $\frac{656/9{,}425}{20/138} = 45\%$ (latest data), yet
  - ▷ Low prevalence of familial WT ($\sim 1.4\%$)
  - ▷ Low recurrence rate in offspring ($< 3\%$)

- 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."

Knudson AG, *Med Pediatr Oncol* **21**:193-198, 1993

Genomic Imprinting and Wilms Tumor



- Expression depends on parental origin
  - ▷ Allelle inherited from Mother switched off (imprinted)
  - ▷ Allelle inherited from Father expressed
- Loss of imprinting (LOI)
  - ▷ Both alleles expressed
- LOI of *IGF2* @ 11p15 observed in $\sim$ 70% WT
- Other genes *paternally* imprinted
  - ▷ *e.g.*, *H19* @ 11p15

Genetic and Epigenetic Evidence for Heterogeneity

- Multiple familial WT genes
  - ▷ *WT1* @ 11p13, *FWT1* @ 17q, *FWT2* @ 19q, ...
- Mutations in *WT1*, *CTNNB1* @ 3p22 and *WTX* @ Xq11
  - ▷ Any 1, 2 (*WT1*+) 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

Miller RW, Fraumeni JF Jr, Manning MD, *N Eng J Med* **270**:922-7, 1964

Denys-Drash Syndrome (DDS)

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

2. Wilms tumor

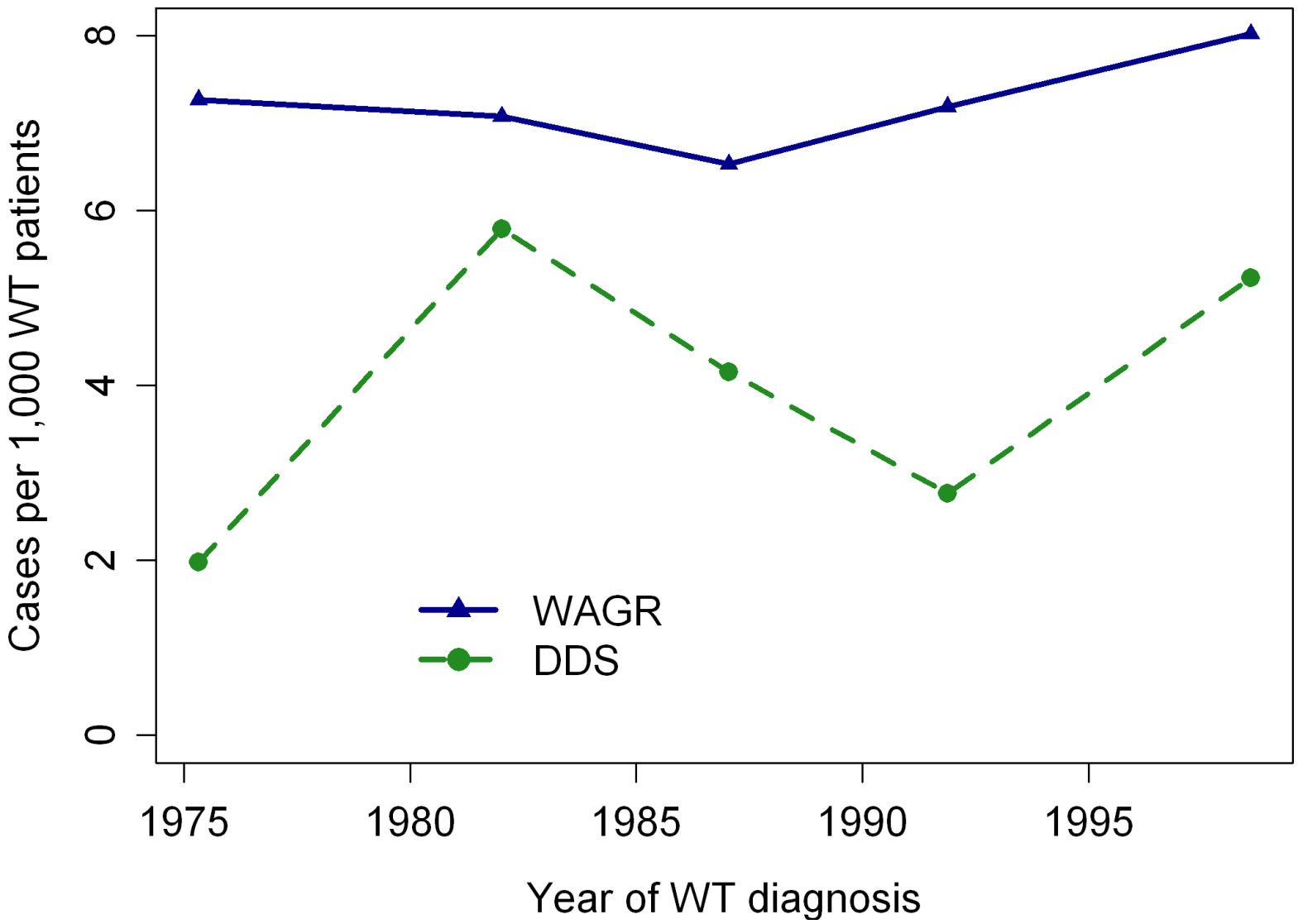
3. Disorders of sexual differentiation ("ambiguous genitalia")

Caused by constitutional point mutations in *WT1*

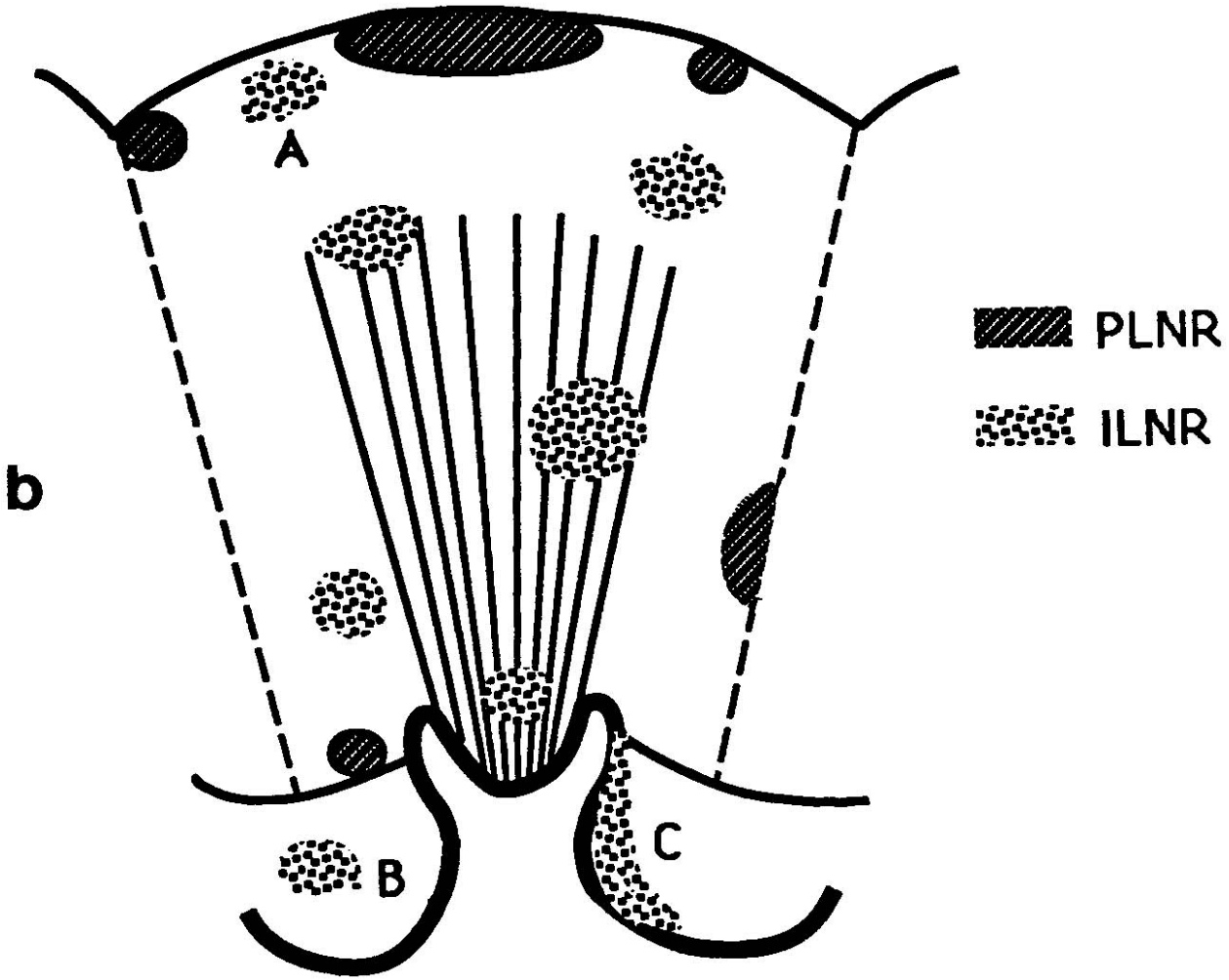
Denys P et al., Archives Françaises de Pédiatrie **24**:729-39, 1967
Drash A et al., Journal of Pediatrics **76**:585-93, 1970

NWTS: Prevalence of WAGR and DDS

Cases per 1,000 WT patients

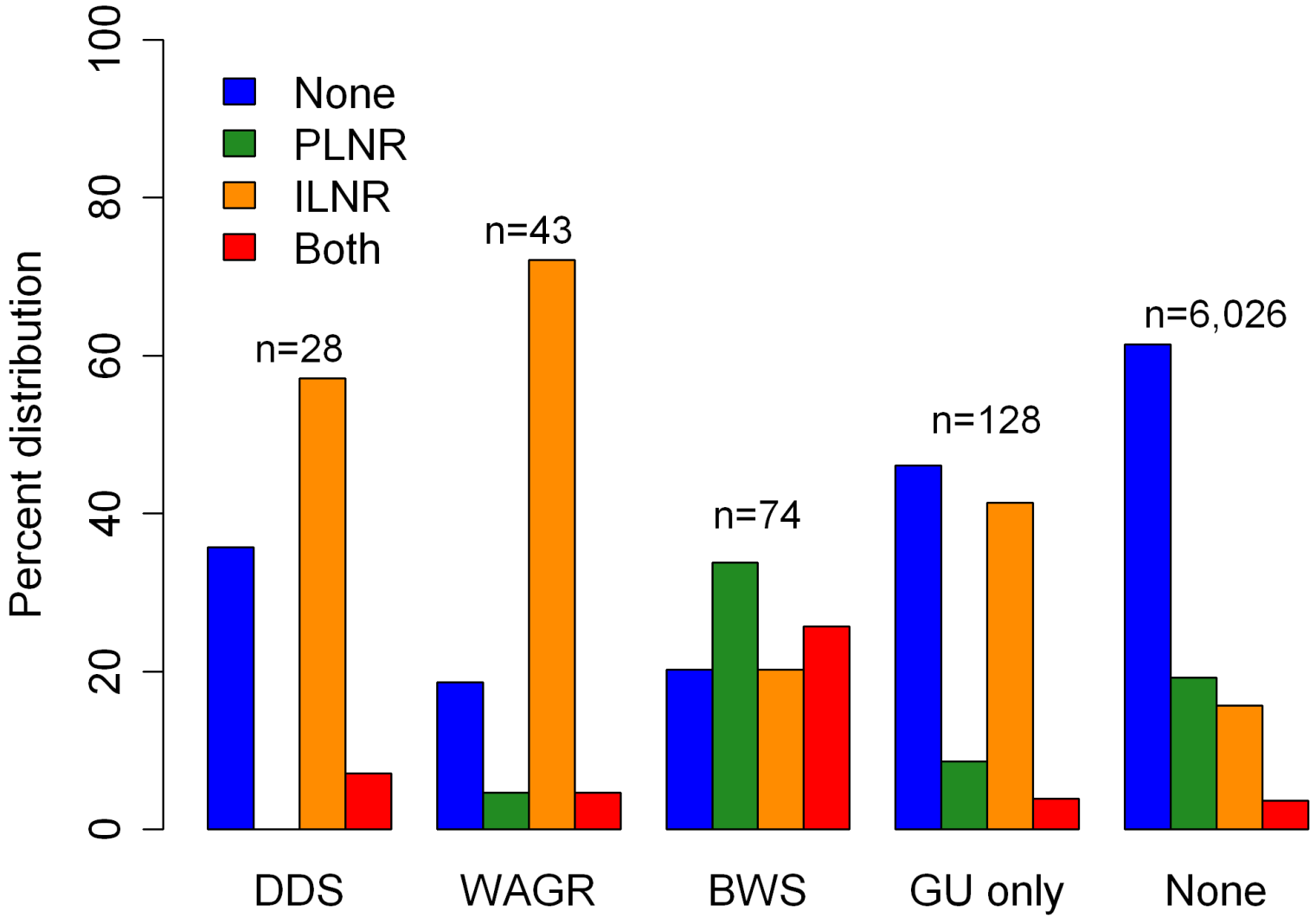


Nephrogenic Rests as Precursor Lesions



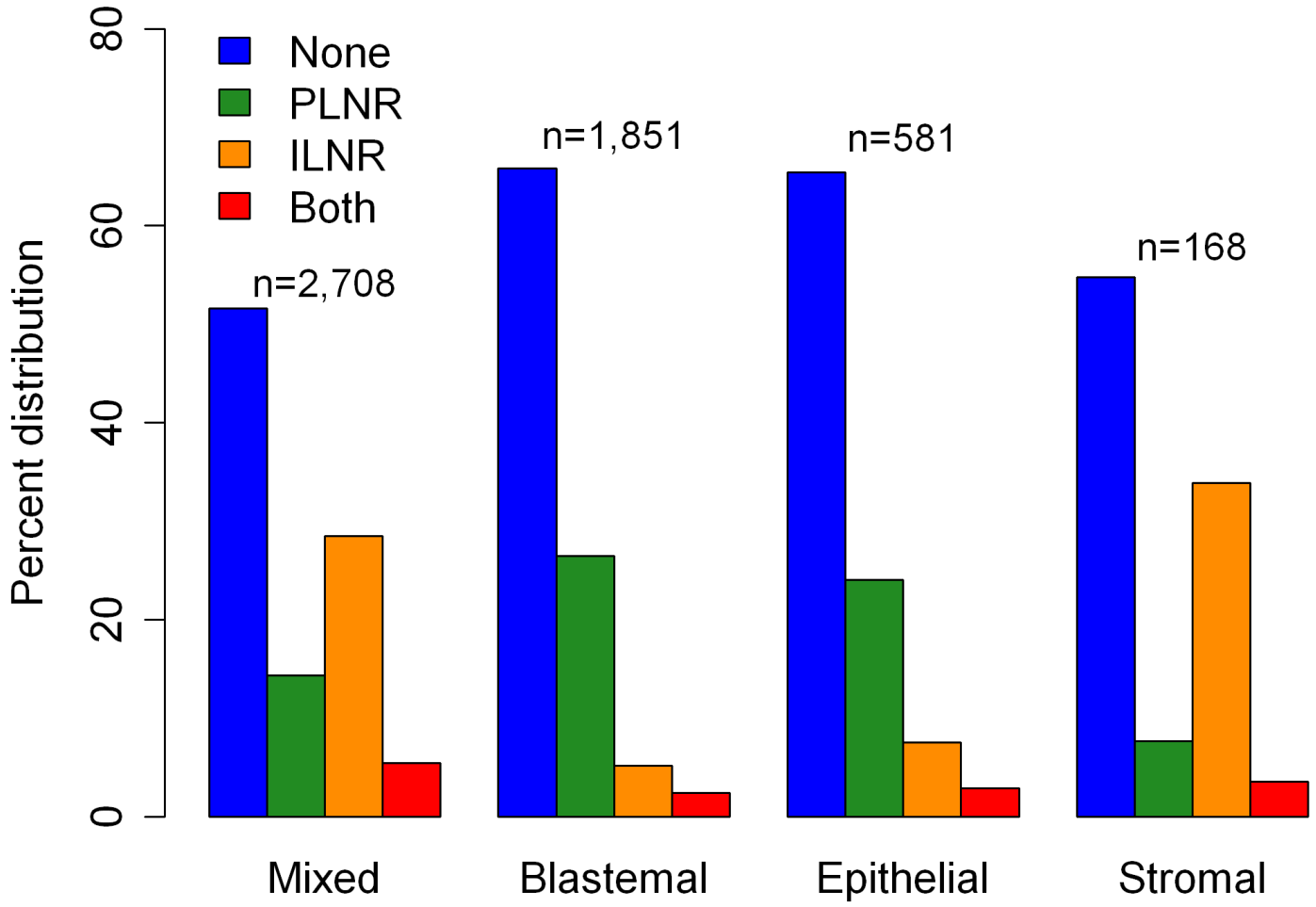
Beckwith, Kiviat, Bonadio. *Pediatr Pathol* **10**:1-36, 1990

Nephrogenic Rests and Congenital Anomalies



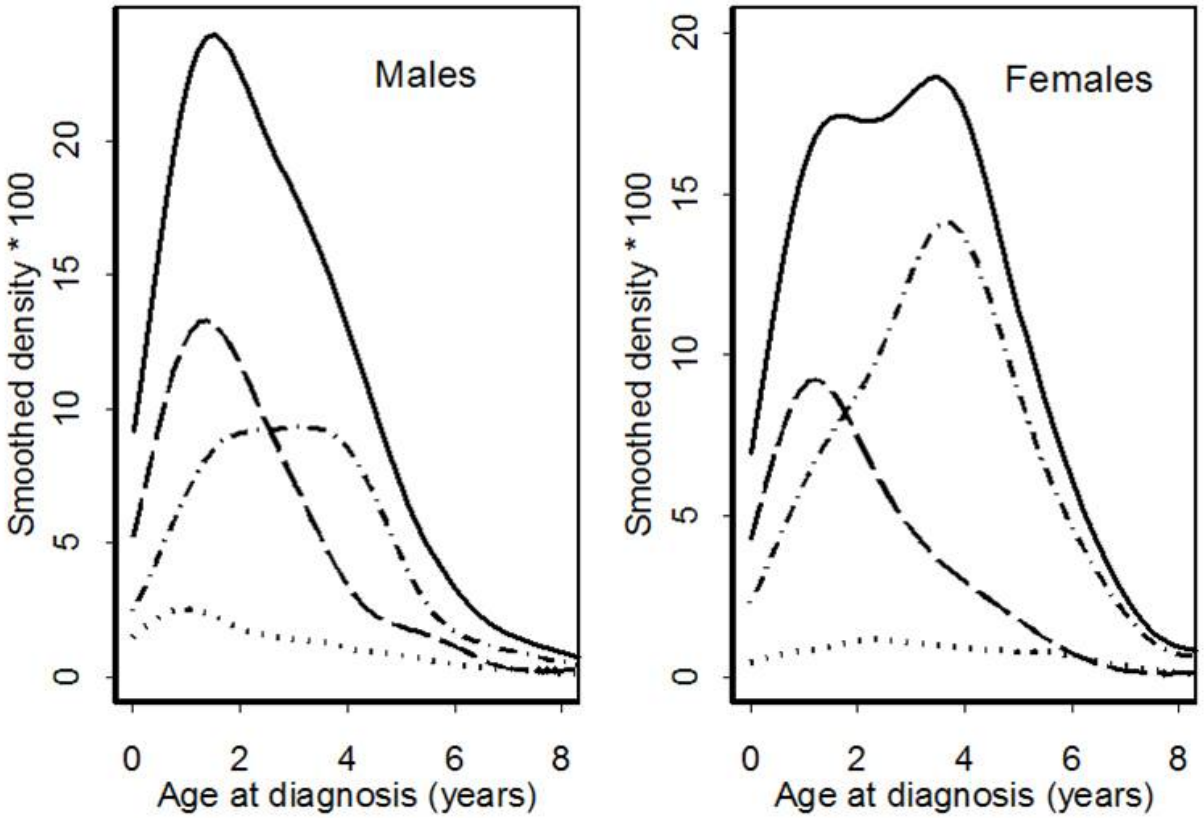
Nephrogenic Rests and Histologic Subtype

Patients with Favorable Histology Wilms Tumor



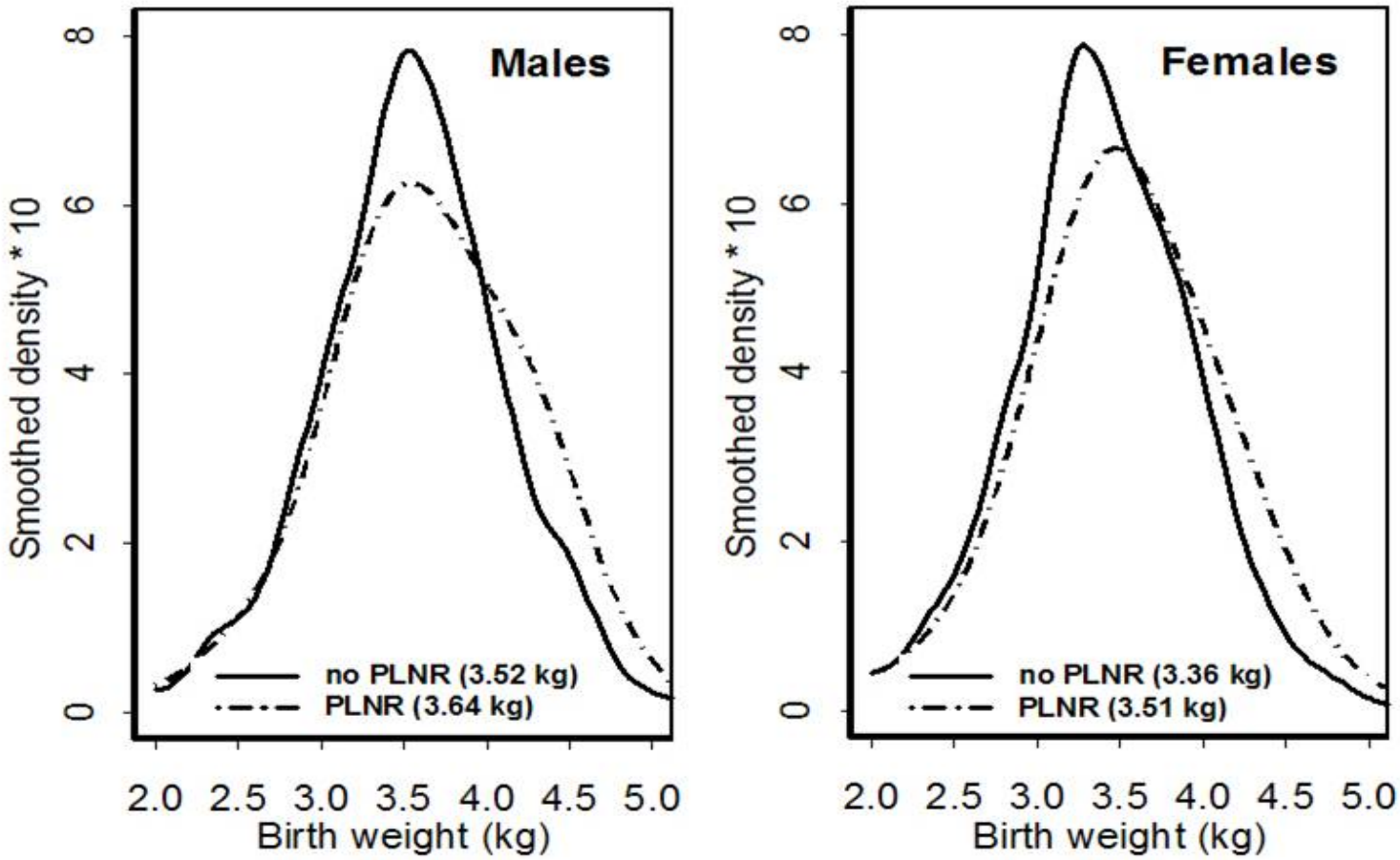
Nephrogenic Rests, Gender and Age

Patients with Multifocal Wilms Tumor



$\cdots$ No rests identified; — — ILNR±PLNR; — · — PLNR only

Nephrogenic Rests, Gender and Birth Weights



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

Breslow, Beckwith, Perlman, Reeve. *Pediatr Blood & Cancer*, 2006

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*)

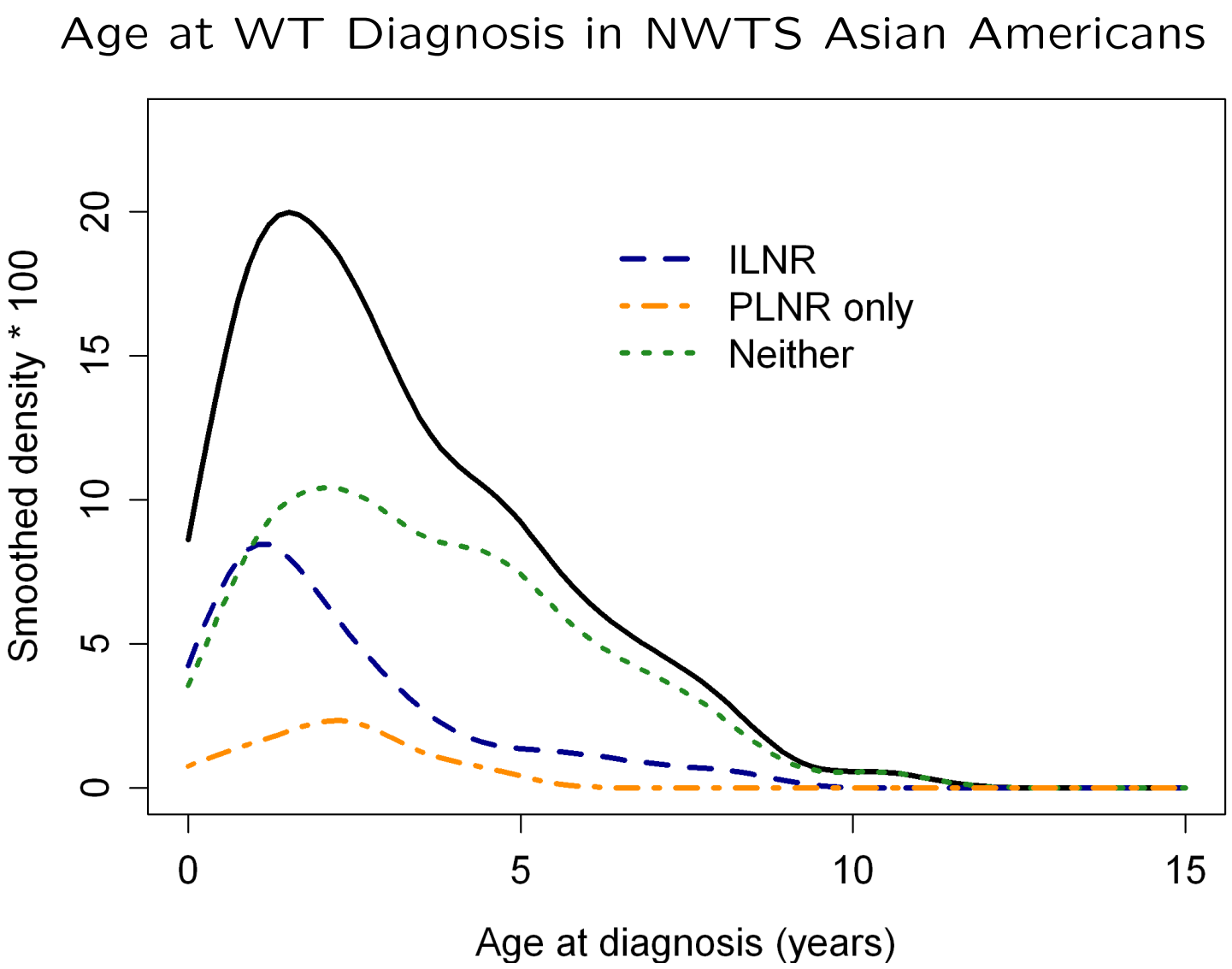
Epidemiology of Wilms Tumor in Asia

- $\sim$ 1/2 incidence rates of Caucasian children
- Earlier ages-at-onset
- (Slight) male predominance

Breslow, Langholz, *Int J Cancer* **32**:703-716, 1983

Breslow, Olshan, Beckwith, Green, *Med Pediatr Oncol* **21**:172-181, 1993

Epidemiology of Wilms Tumor in Asia



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

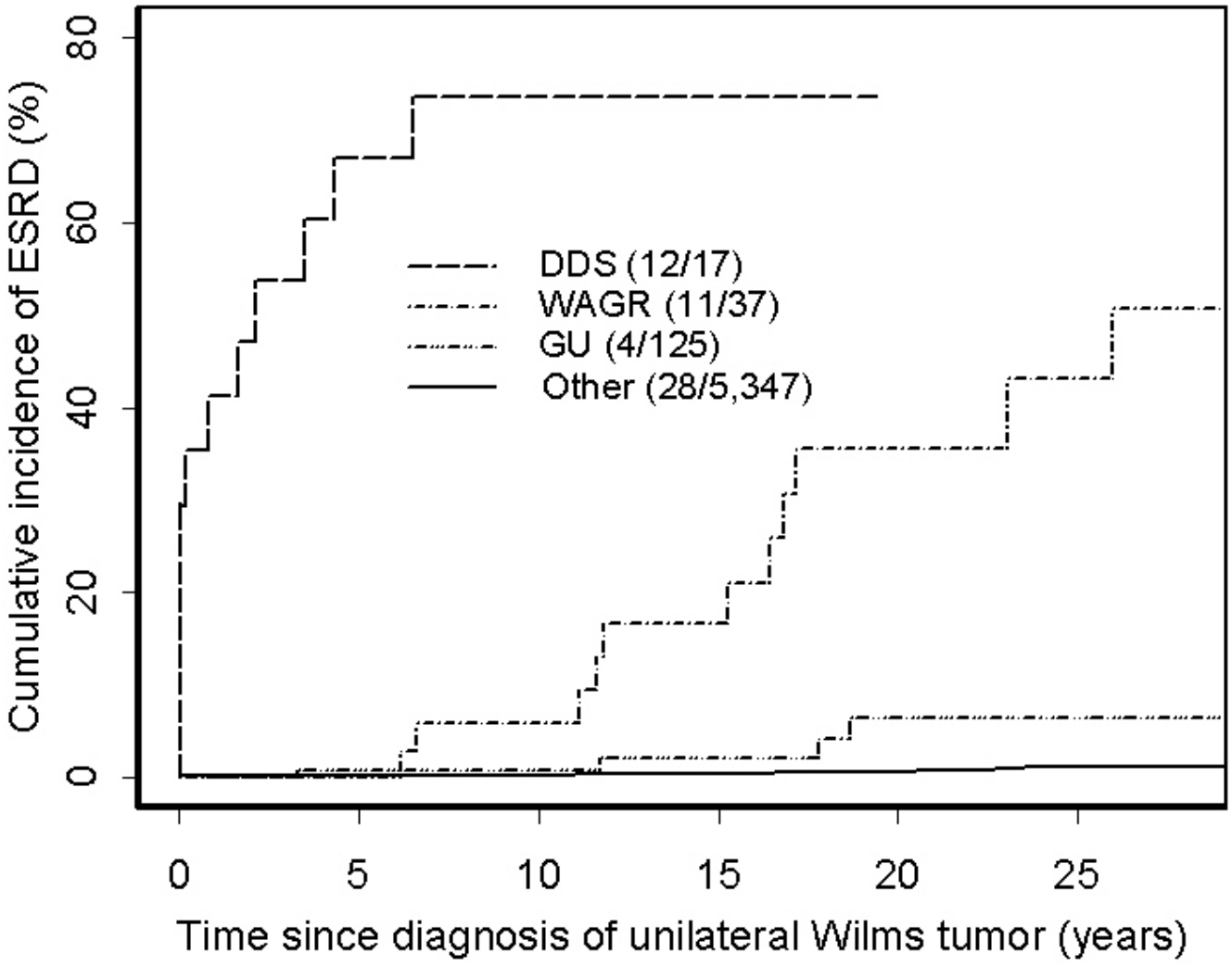
- NWTS 1-4 pts from U.S., complete baseline data
  - ▷ Cohort I: 5,526 pts with unilateral WT at Dx
  - ▷ Cohort II: 384 pts with bilateral WT (66 metachronous)

- 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

Breslow, Collins, Ritchey *et al.*, *J Urol* **174**:1972-1975, 2005

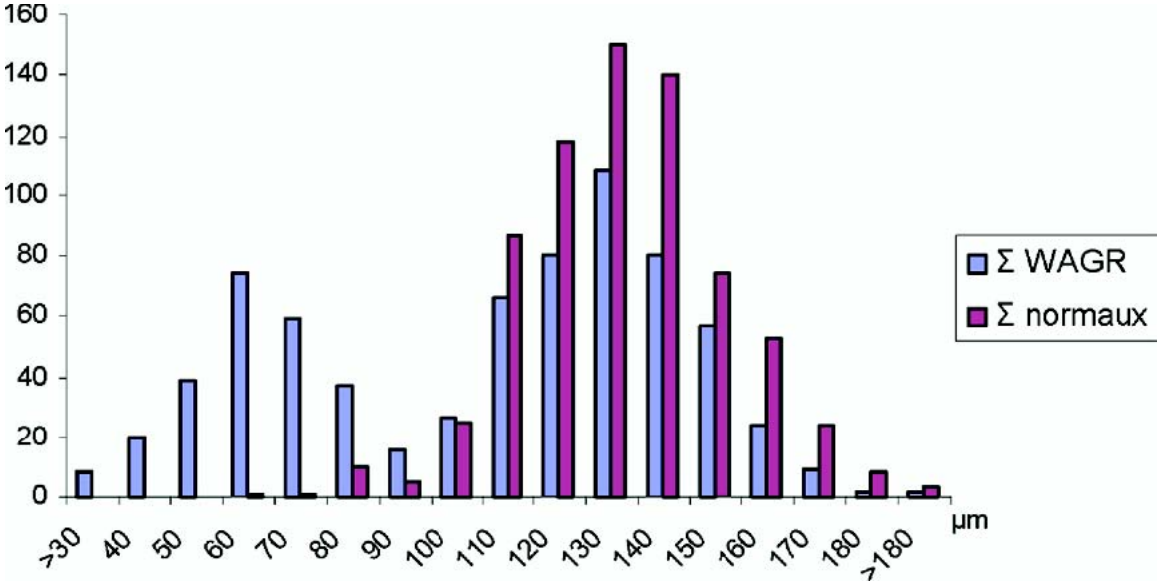
Cumulative Incidence of ESRD: Unilateral WT



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

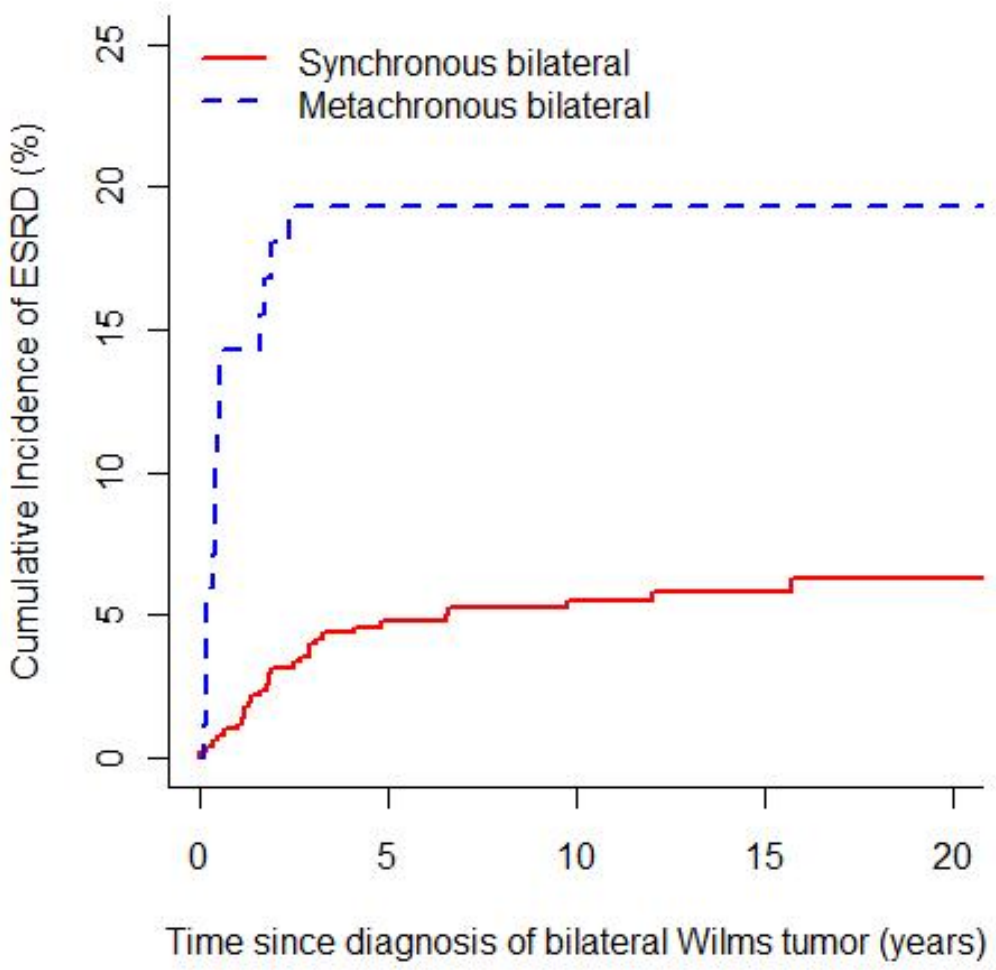
Lange, Peterson, Takshima *et al*, *J Urol* **186**:378-86, 2011

ESRD Risk Factors Study: A priori Hypotheses

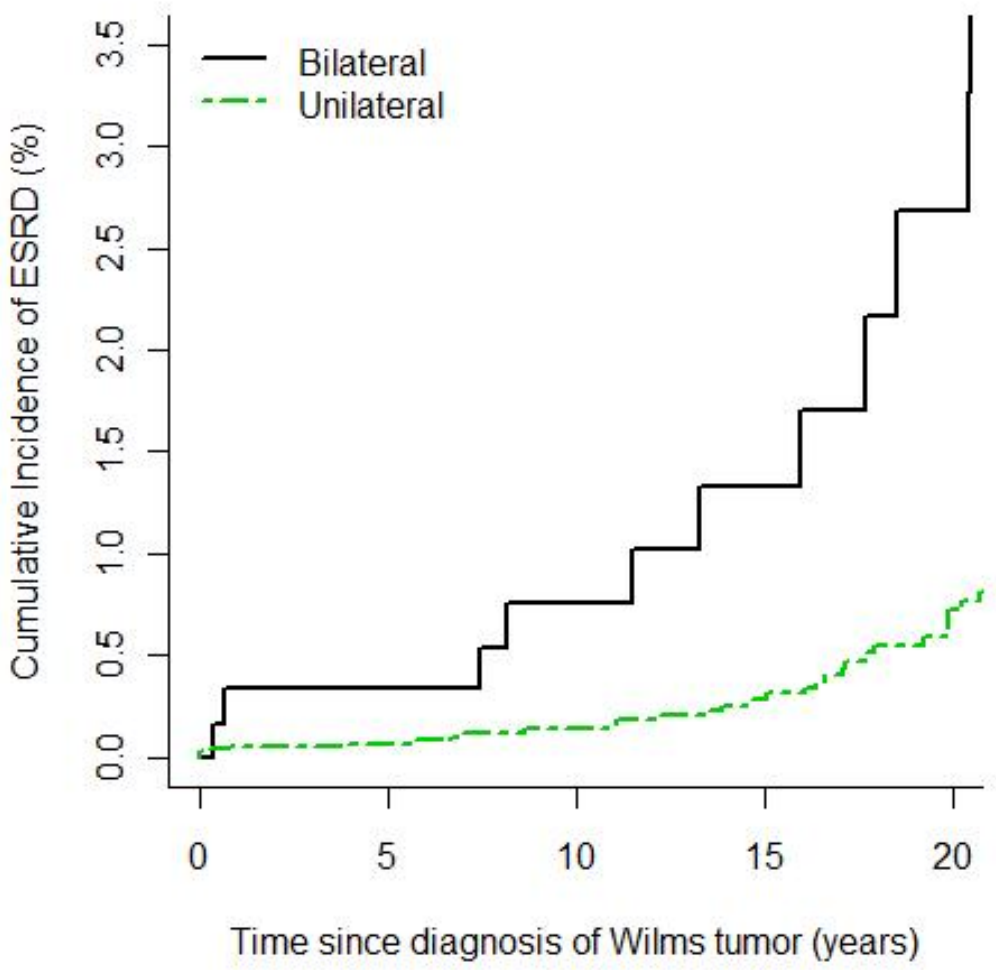
- Patients with *metachronous* bilateral disease have higher rates of ESRD due to progressive bilateral WT than those with *synchronous* bilateral disease
  - ▷ Bilateral at diagnosis $\Rightarrow$ renal sparing surgery
  - ▷ Wilms tumors that develop while patient is on (or shortly after completion 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

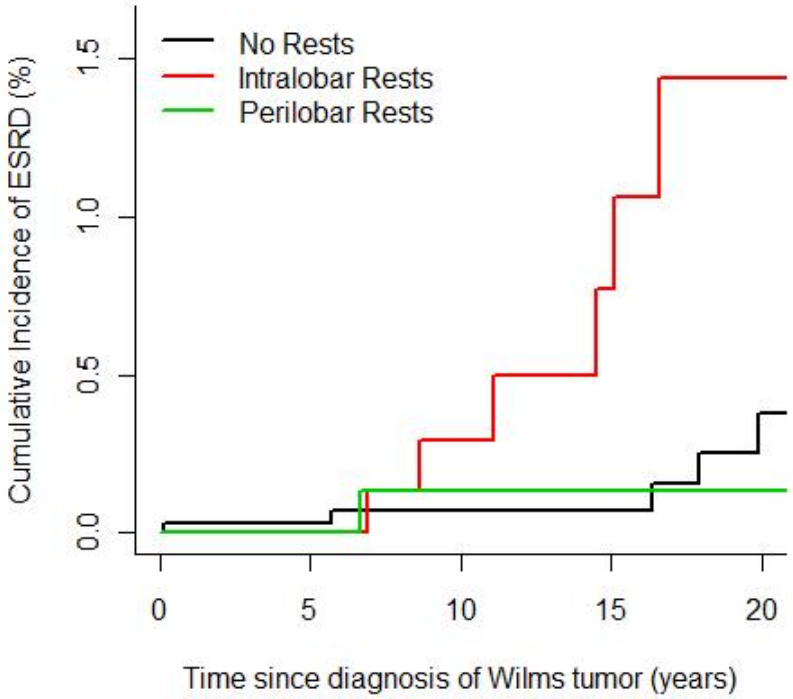
Cumulative Incidence of ESRD Due to Progressive Bilateral Wilms Tumor

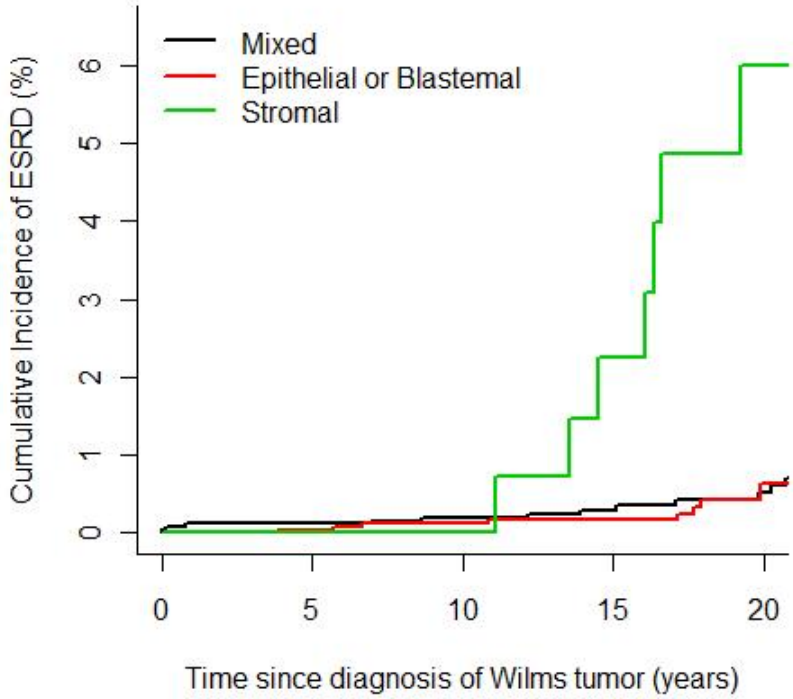


Cumulative Incidence of ESRD Due to Chronic Kidney Disease



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

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

* 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 R_x
  - ▷ 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

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Thank You, Dan and Audrey!

Married Feb 1, 2005