The Northern California Childhood Leukemia Study (NCCLS):

10 Years of Experience in Environmental and Genetic Epidemiology

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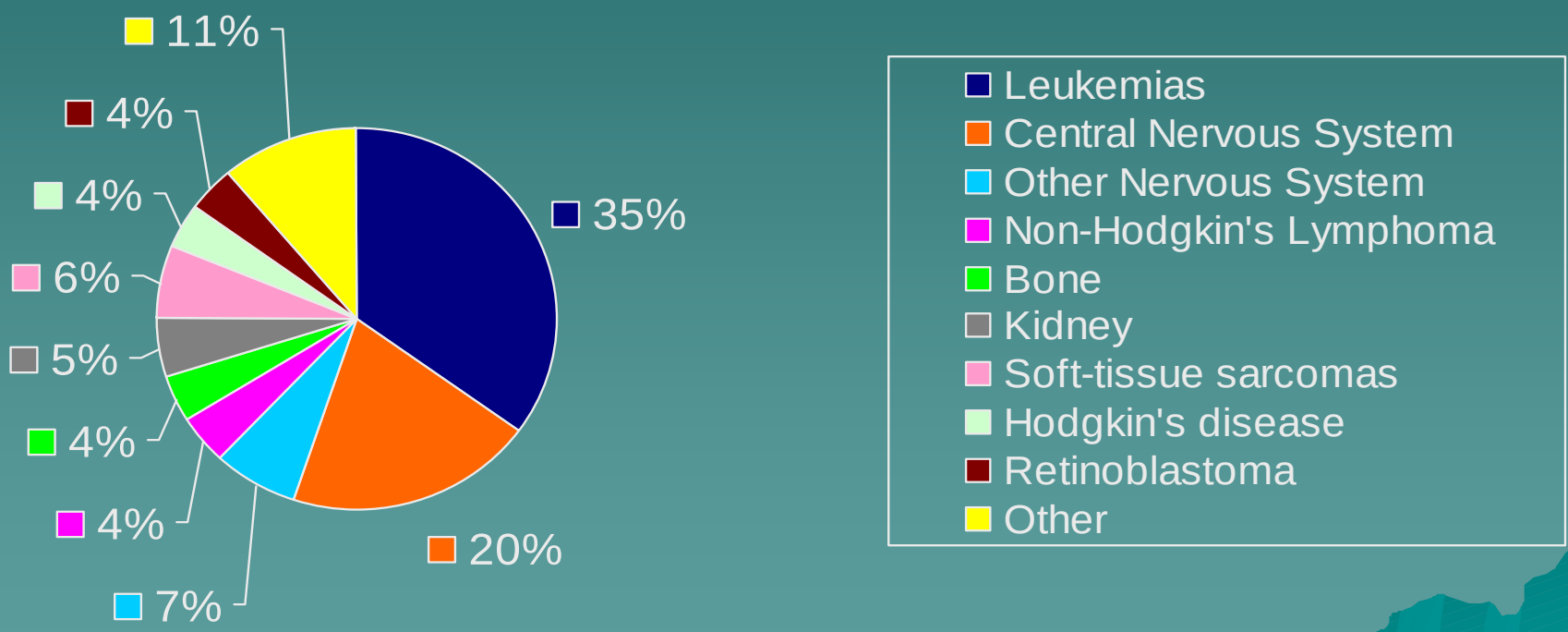
NIEHS Superfund Basic Research Program

Annual Meeting - January 12-13, 2006

NCCLS

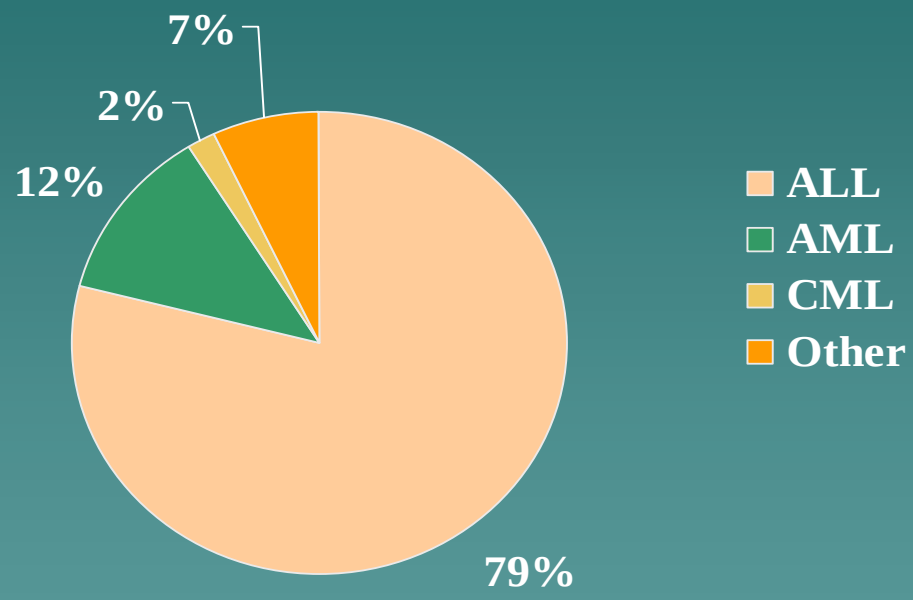
Childhood Cancers (ages 0-14)

Percent distribution by type



Childhood Leukemia

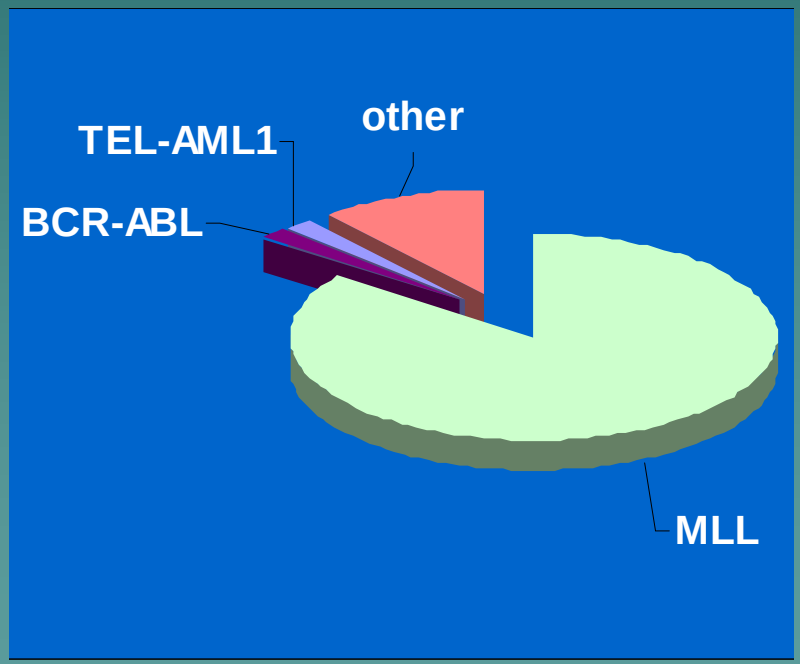
Heterogeneous disease with 4 major histologic subtypes



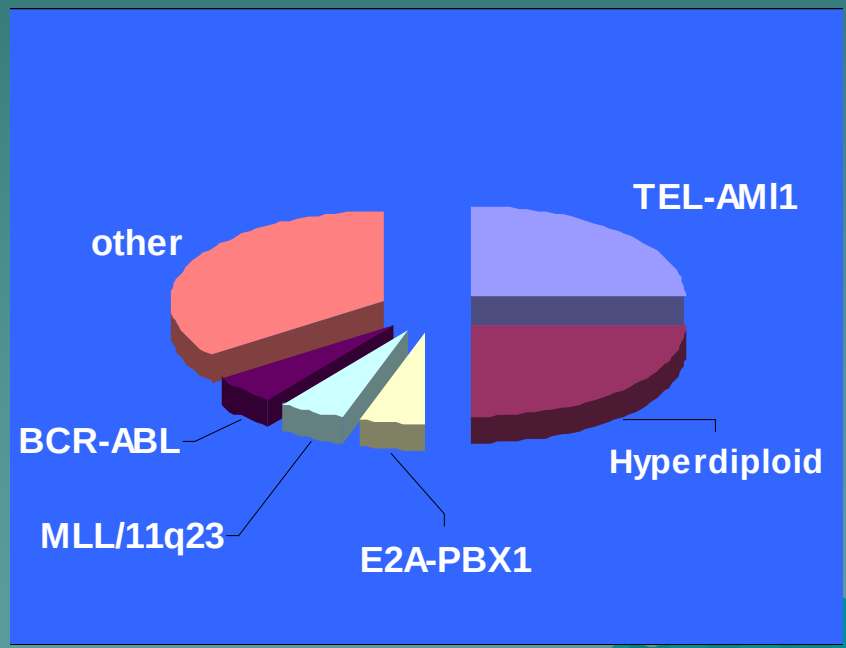
Source: Cancer in California, 1988-1991

Molecular Subsets of ALL

INFANTS



CHILDREN



Facts About Childhood Leukemia

Approximately 2,500 new cases per year among children under age 15 years in the U.S.

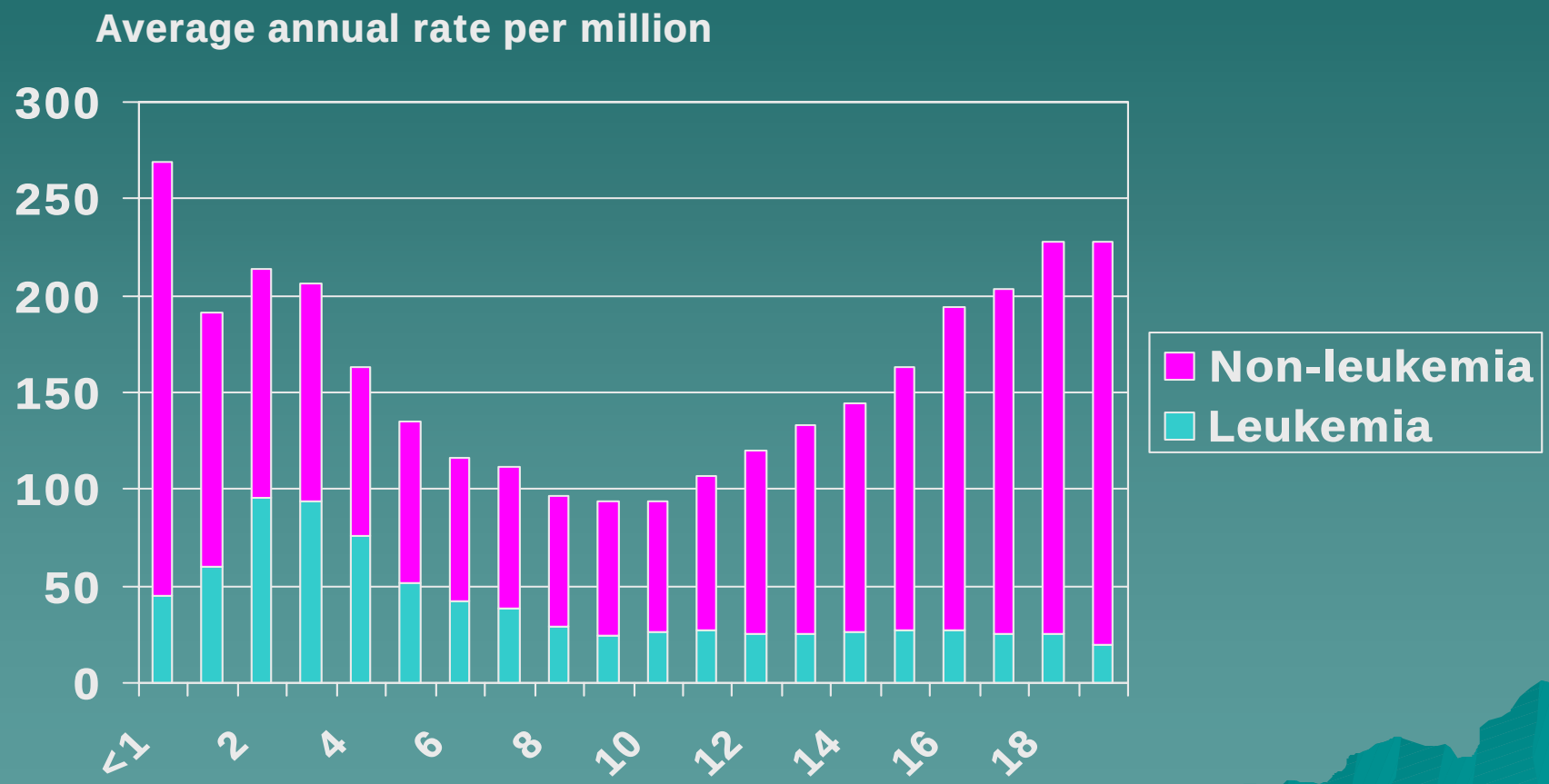
Highest incidence rates in

- Whites
- Hispanics
- Males

Peak incidence of leukemia at age 2-5 years

Total childhood cancer age-specific incidence rates by leukemia vs. non-leukemia

(all races, both sexes, 1986-94)



Causes of Childhood Leukemia

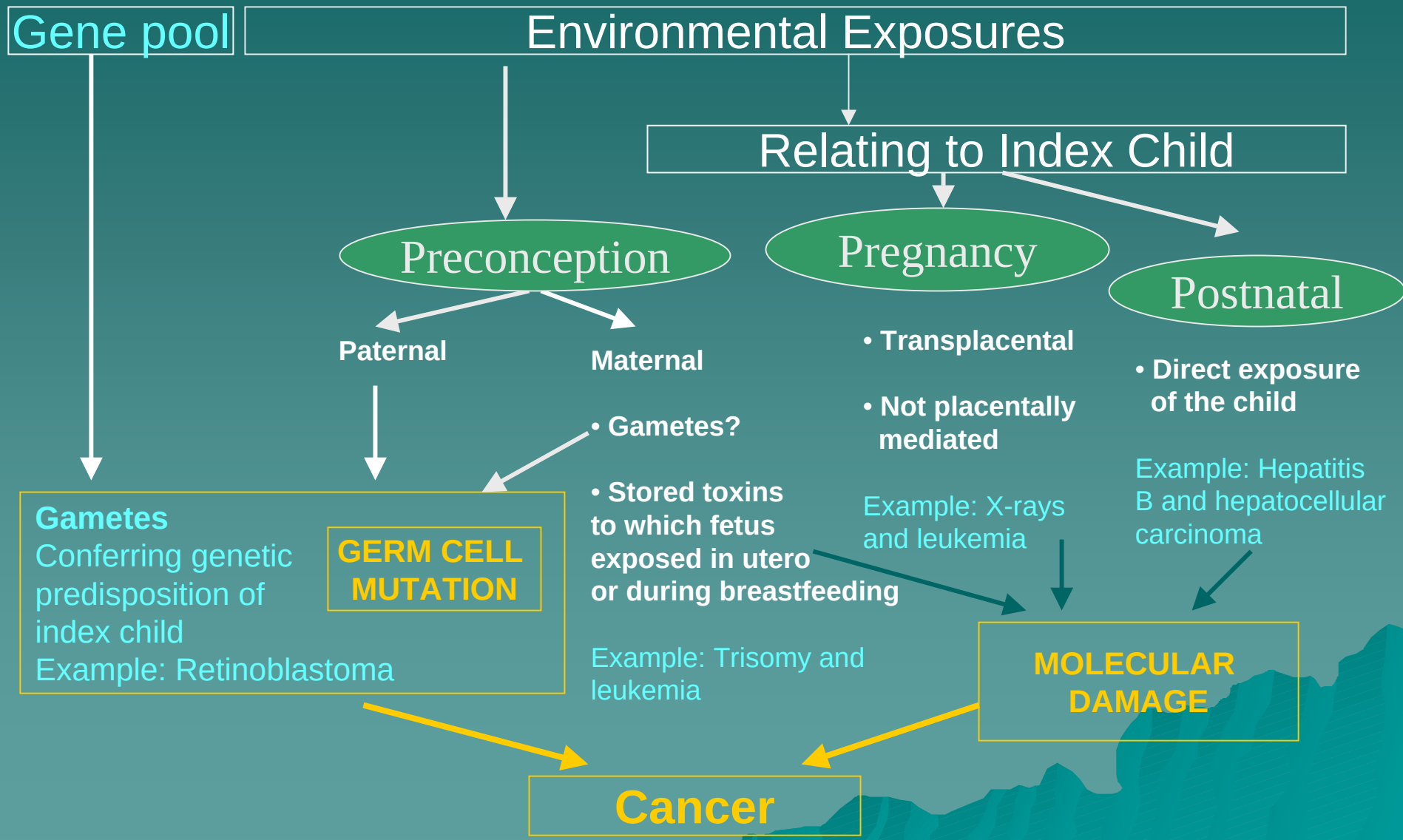
- The causes of 90% of childhood leukemias are unknown

- Established risk factors account for only 10%
  - genetic conditions (e.g., Down syndrome)
  - ionizing radiation (*in utero* & postnatal)
  - chemotherapeutic agents

Suspected Risk Factors for Childhood Leukemia

- Residential chemical exposures of parents and/or child
- Chemicals to which parents have been exposed at work
- Tobacco smoke
- Viral infections
- Dietary exposures, especially micronutrients, of parents and/or child
- Non-ionizing radiation exposure of parents and/or child

Schematic Framework for Considering Cancer Etiology in Children



Two-hit model

- Gene rearrangements
  - Hallmark of CL
  - But they are not always sufficient for CL → may be the first "hit"
- One or more additional "hits" may be needed
  - Child's genetic susceptibility
  - *In utero* exposures (incl maternal effects)
  - Post-natal exposures

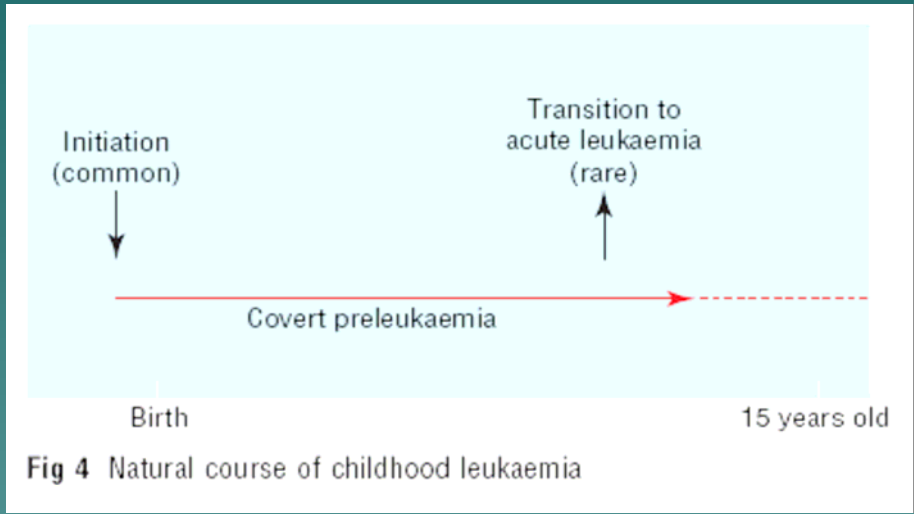


Fig 4 Natural course of childhood leukaemia

From: Greaves M, *BMJ*, 2002

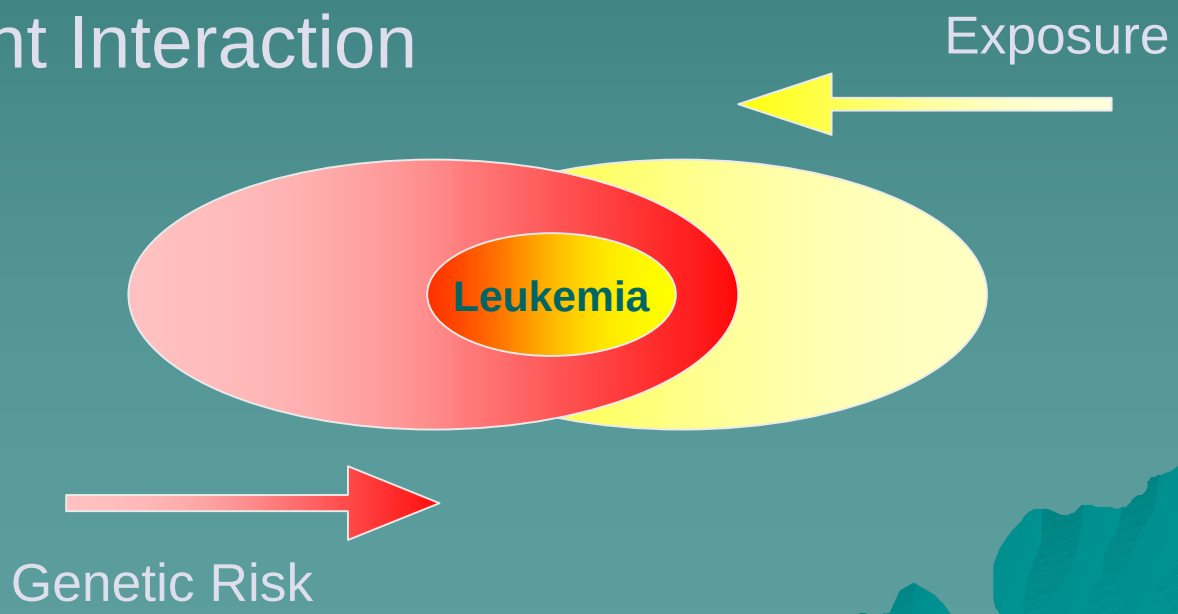
The NCCLS Objectives

- Examine the relationship between environmental exposures and childhood leukemia
  - Pesticides & chemicals in households & drift from residential areas and parental workplaces, tobacco smoke infectious agents, and diet
  - **During critical periods of the child's development**
  - For overall and major molecular leukemia types
  - For White non-Hispanics and Hispanics
- Explore modification of risks by metabolic polymorphisms

Genetics and Environmental Risk Factors in Childhood Leukemia

- Environmental Factors
- Genetic Factors
- Most likely a Gene-Environment Interaction

Benzene
Infection
Diet

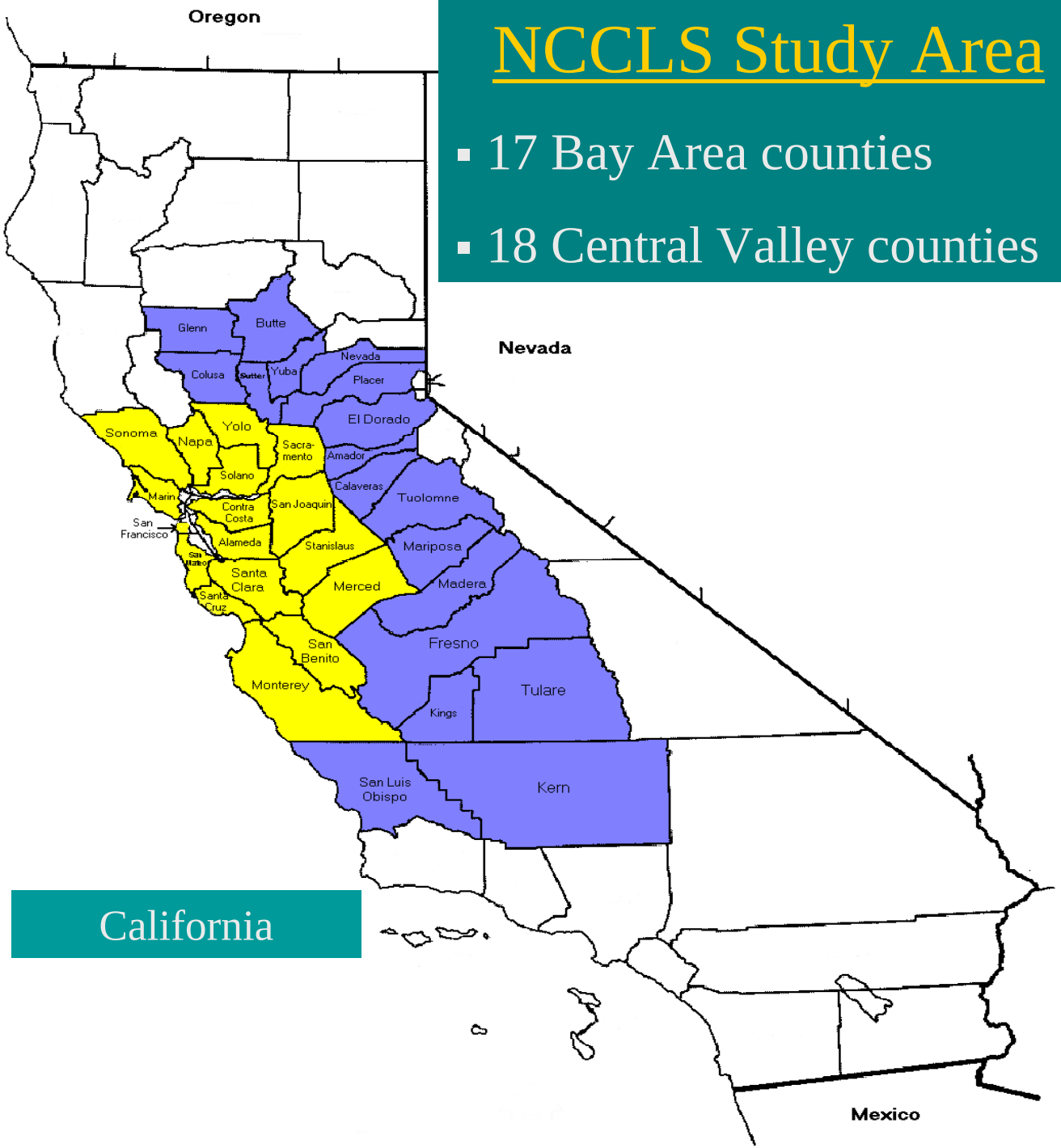


The NCCLS Design

- Population-based case-control study
- Started in 1995 – End of enrollment in 2008
- Network of 9 pediatric oncology centers in 35 counties in Northern and Central California
- Inclusion of Hispanic population (47%)
- Multi-disciplinary team
  - Pediatric oncologists, epidemiologists, molecular biologists, nutritionists, toxicologists, and industrial hygienists
- Primarily funded by the National Institute Environmental Health Sciences

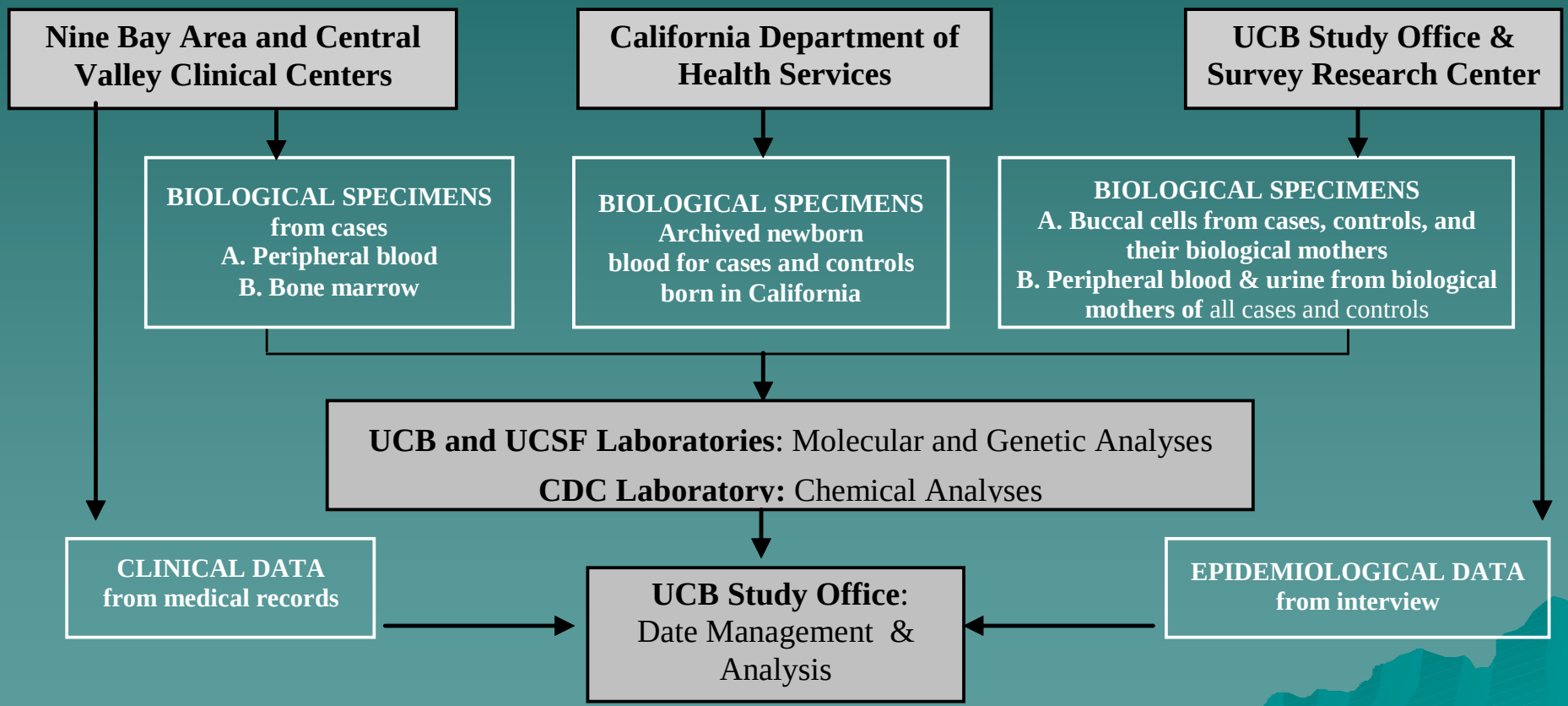
NCCLS Study Area

- 17 Bay Area counties
- 18 Central Valley counties



California

Collaborating Institutions of the NCCLS



NCCLS Case Eligibility Criteria

- New diagnosis of leukemia
- 0-14 years old
- Biological parent speaks English or Spanish
- No previous cancer diagnosis
- Resident of study area at time of diagnosis

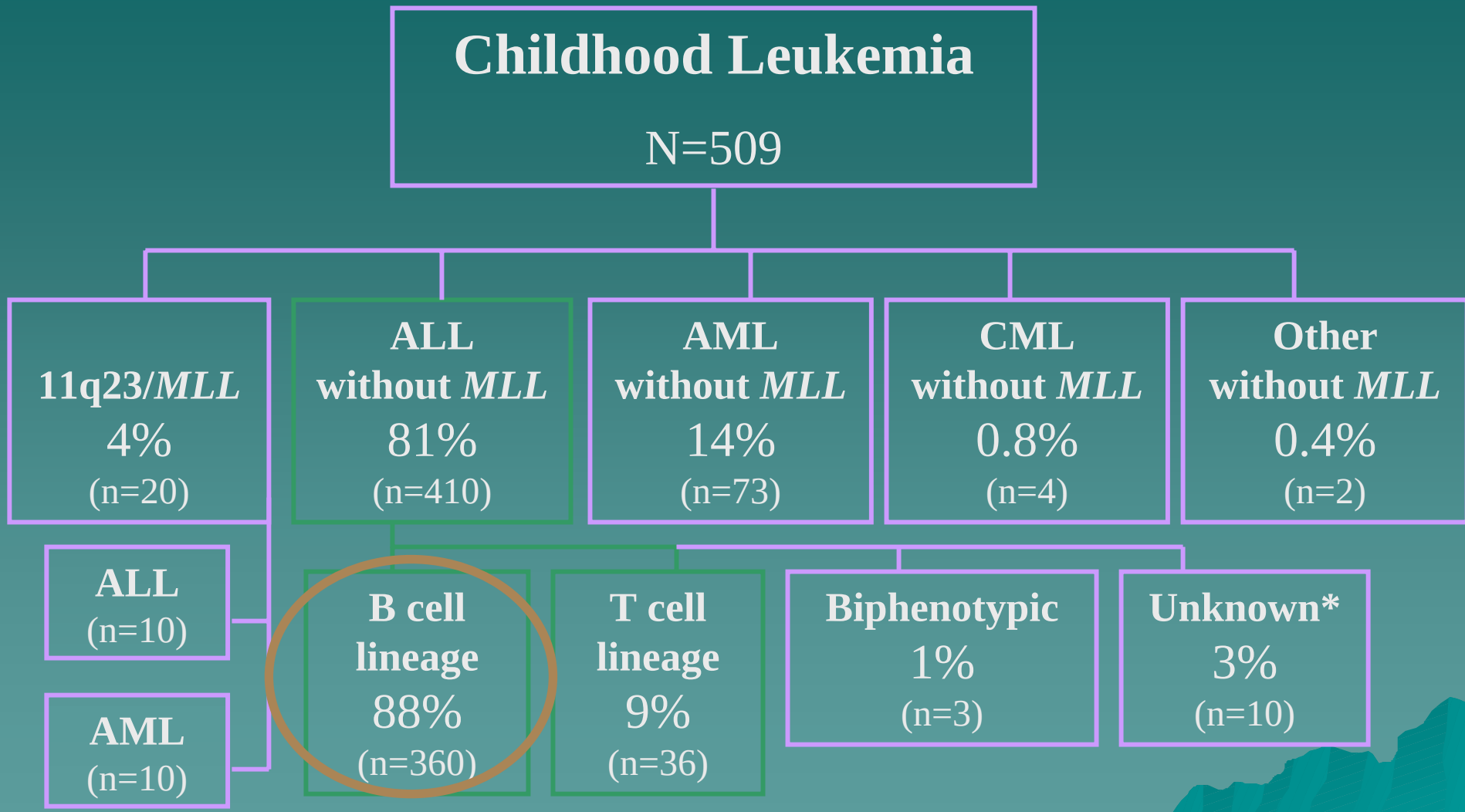
Case Enrollment

- Incident cases rapidly ascertained within 48 hrs
  - Obtain informed consent
  - Collect pre-treatment blood and/or bone marrow specimens within 72 hours in 86%
- NCCLS identified over 88% of incident leukemia cases, compared with California Cancer Registry data (1997-1999).

Case Participation

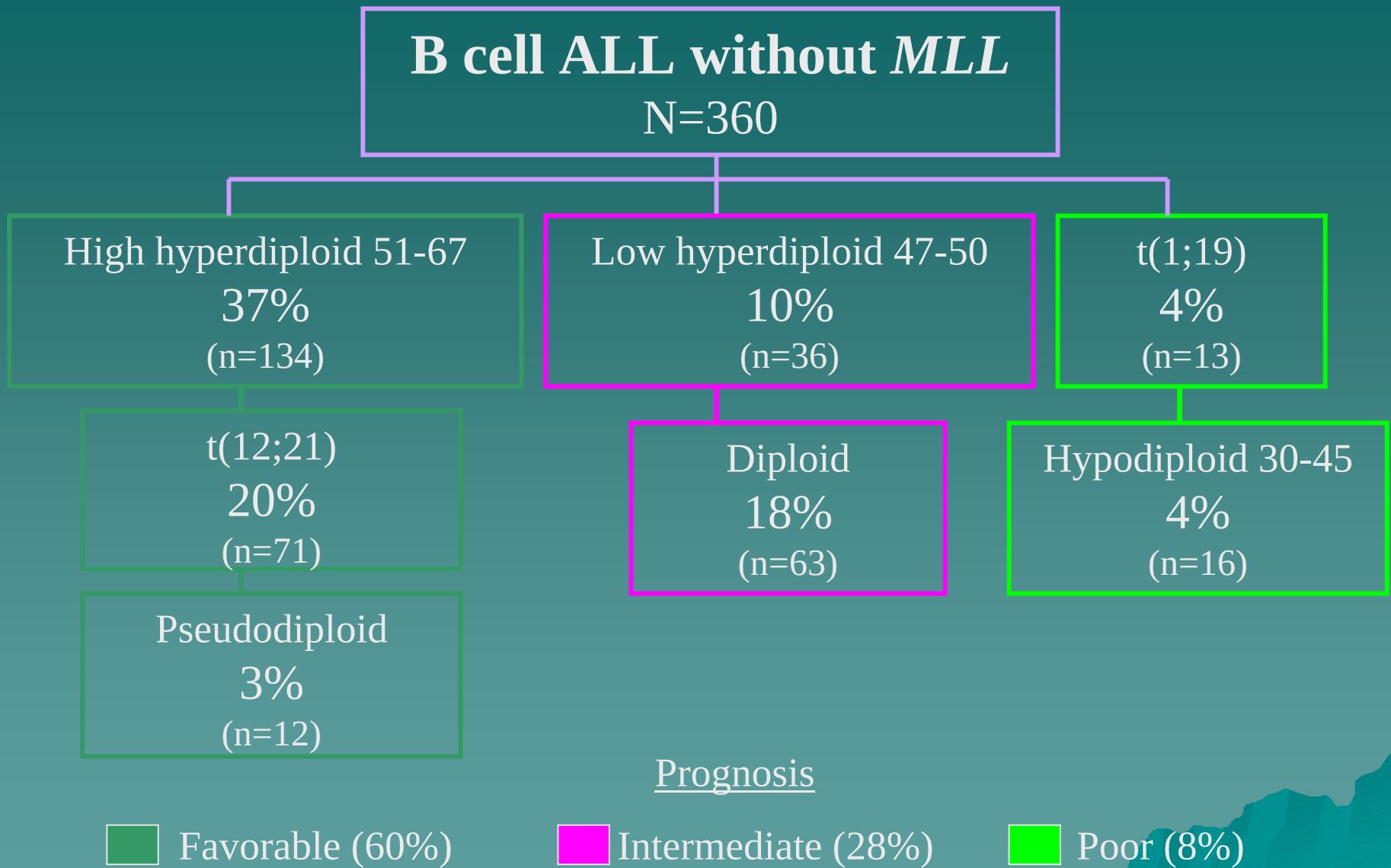
- As of November, 2005, 1243 cases have been ascertained.
- Of these, 960 (77%) are eligible to participate.
- 829 (86%) eligible patients have consented to participate, and 678 have completed interviews.

Cytogenetic Classification



*missing immunophenotype information

Cytogenetic Classification



Not shown: cases with unknown prognosis (n=15)

NCCLS Control Selection

- Concurrent to case ascertainment
- Achieved using **California Birth Registry** and electronic tracing technologies
- Individually matched to case by:
  - Date of birth, gender, maternal race, Hispanic status, mother's county of residence at child's birth
  - 1 or 2 controls per case

Control Participation

- The number of searches conducted for each participating control ranges from 1 to 16, with an average of 2.7.
- Approximately 66% of participating controls are first choice or “ideal” controls.
- Assess the representativeness of participating controls to the source population, by comparing socio-demographic characteristics between the participating and non-participating controls

Collection of Interview Data

- In-person computerized assisted interview
  - Biological parents (mostly mothers)
  - English or Spanish
- Comprehensive questionnaire
  - Detailed time-specific exposure assessment
  - Mother and child's diet
  - Daycare attendance and childhood infections
  - Residential history (=> geocoding)
  - Parental smoking
  - Parental occupation
    - Job titles
    - 19 task-specific questionnaires adapted from NCI job modules (no surrogate interviews)
  - Household chemical use

Collection of Biospecimens

- Blood and bone marrow specimen: 86% cases
  - Using proteomics to classify leukemia into molecular subgroups
  - RAS mutation
- Buccal cells in case & control children and their mothers: 98%
  - Genetic polymorphisms
- Archived Newborn Blood specimens (Guthrie cards) for case & control children: 85%
  - Backtracking to birth of chromosome translocations in cases: t(12;21), t(8;21), t(15;17), inv (16)
- New: maternal blood/urine specimens
  - Collaboration with CDC-National Center for Environmental Health for analyses of chemicals and folate levels
  - Use of protein adducts as a biomarker of exposure

Genotyping in the NCCLS

- DNA extracted from buccal cells and ANB specimens and amplified to permit assaying of 1000's of SNPs
  - Examine effects of child's own genetic susceptibility
  - Examine *in utero* effects related to maternal genes
- Focus on candidate genes encoding enzymes involved in important pathways (e.g., protecting from environmental insults, cell growth and regulation):
  - Xenobiotic metabolism and transport enzymes (exogenous substances, including chemicals, pesticides, benzene, pollutants)
  - Metabolism of nutrients including folate, other vitamins, growth factors
  - Antioxidant enzymes
  - DNA repair enzymes
  - Immune function

Genotyping in the NCCLS

- Preliminary genotyping on ~20 SNPs in a limited group of samples
- Plan to type all samples (Illumina)
  - ~1000 cases, ~1500 controls
  - ~200 genes
  - Birth mothers (~2500)
  - Case fathers
- Goals
  - Child susceptibility
  - Maternal-child effects
  - Genetic transmission to case children from mothers and fathers

Collection of Environmental Samples

- Follow-up-visit within 3 to 9 months
  - Children age ≤7 yrs; same residence as diagnosis or reference date
  - Reliability study on household chemical use
  - Air, dust, and window wipe sampling

Multi-Step Approach to Characterize Exposure to Pesticide

ELIGIBILITY

CHILD AGE < 8 YEARS AND LIVING AT CURRENT RESIDENCE SINCE REFERENCE DATE

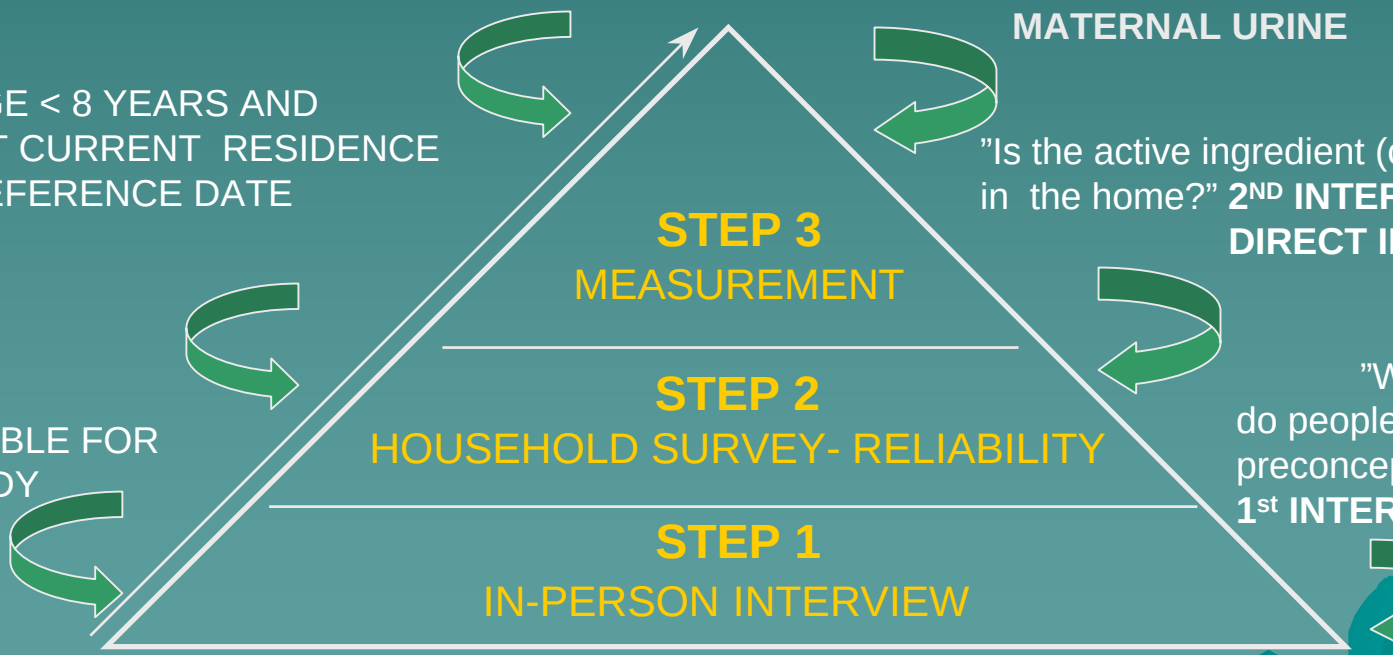
ALL ELIGIBLE FOR THE STUDY

RESEARCH QUESTIONS

”What levels of the relevant compounds are present in the home environment currently?”
DUST & WINDOW WIPE SAMPLING
MATERNAL URINE

”Is the active ingredient (compound) present in the home?” **2ND INTERVIEW and DIRECT INVENTORY**

”What products do people report using from preconception to age 3 years?
1st INTERVIEW



Environmental Home Sampling

- Commenced in 2002
- About 50% of case and control families
- Collection of dust samples
  - Collaboration with NCI
  - 380 samples as of October 31, 2005
  - Current analysis of pesticides, polychlorinated biphenyls, & nicotine
- Collection of air samples
  - 355 samples as of October 31, 2005
  - Current analyses of benzene & toluene
- Target = 489 case and control homes

Dust sample collection during home visit

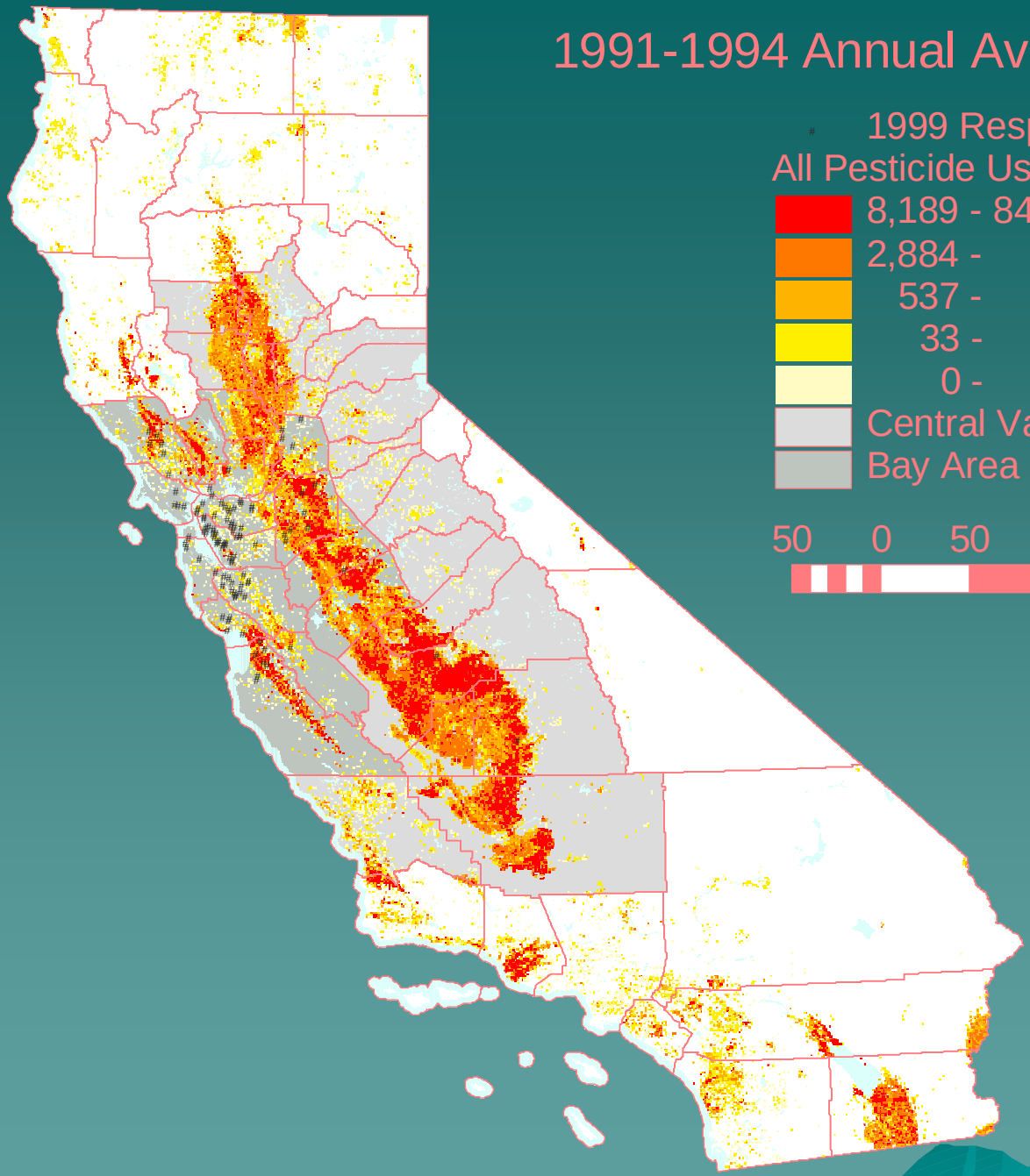
High Volume Surface Sampler (HVS3) vacuum

Pesticides

Multiple Sources of Exposure

- Environmental
  - Parental workplace
  - Drift from nearby agricultural areas
  - Home use
  - School use
- Dietary
  - Water
  - Food
  - Breastfeeding

1991-1994 Annual Average Pesticide Use

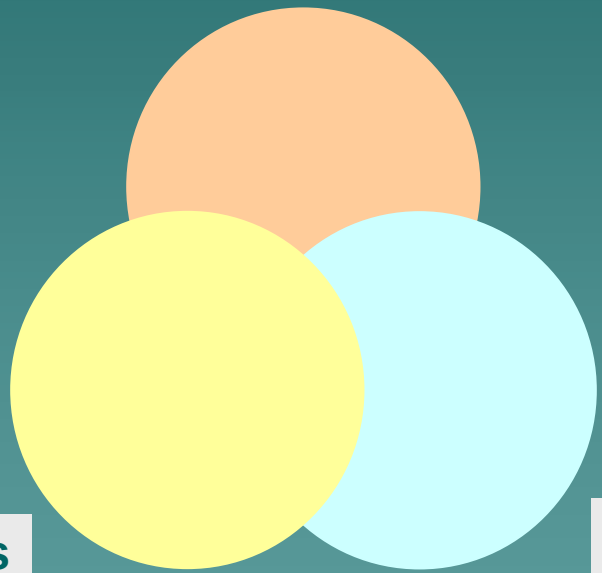


Previous Studies of Childhood Leukemia and Pesticide Exposure

- Agricultural use
  - Ecologic & case-control studies in California (Reynolds, 2002 & 2005)
    - No association with childhood cancers
    - Suggestion of 50% increased risk of leukemia in children exposed to propargite, an insecticide used in orchard & vineyards
- Parental occupations
  - Several studies showing increased leukemia risk, but questionable exposure assessment
  - Increased incidence of cancers and lymphomas in children of the pesticide applicators enrolled in the NCI Agricultural Health Cohort Study (Flower K, 2004)
- Home use
  - Canadian (Infante-Rivard C, 1999) and French (Menegaux F, 2005) studies with similar design as the NCCLS reported increased risks with use of home and garden insecticide during pregnancy and childhood

Model for Pesticide Exposure Assessment in the NCCLS

Self-reports
Residential history
Pesticide use at home
Parental occupational history



Home dust samples

GIS attributes
Linkage to the Pesticide Use Registry, CA

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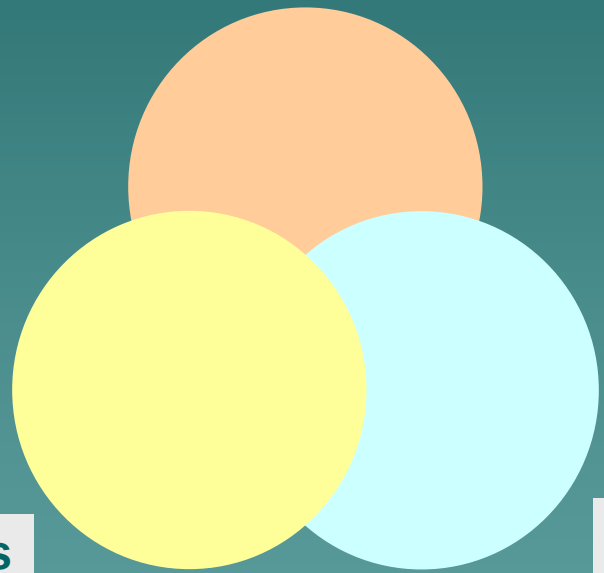

Home dust samples

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Pesticide Exposure and Risk of Childhood ALL

(162 Cases and 162 Controls)

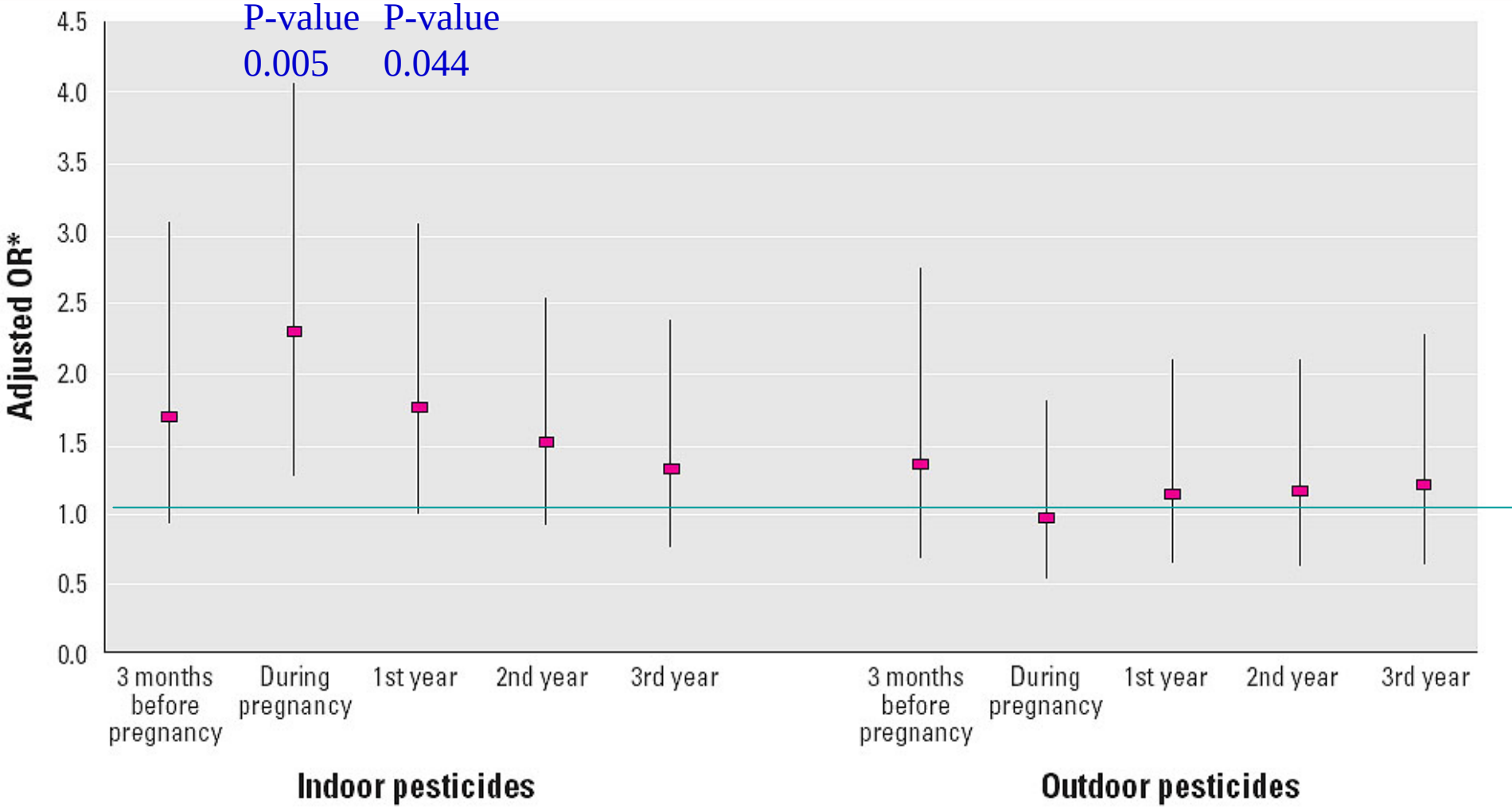

Figure 1. Indoor and outdoor pesticide exposures and the risk of childhood ALL. The boxes are estimated ORs; vertical bars reflect upper and lower limits of 95% CIs.

*Adjusted for annual household income.

Source: Ma X, et al. Environmental Health Perspectives. v.110, no.9, September 2002

Pesticide Use From Preconception up to 3 Years Post-natally in 382 Children With Leukemia and 482 Controls

Type of exposure	Cases/controls	OR[1]
Combined exposures		
Indoor insecticides	299/353	**1.5 (1.1-2.1)**
Outdoor pesticides	136/171	**1.2 (0.9-1.7)**
Outdoor herbicides	159/189	**1.5 (1.1-1.9)**
Selected individual exposures		
Professional pest control	110/122	**1.4 (1.0-2.2)**
Professional lawn services	74/73	**1.6 (1.1-2.3)**
Insecticides	241/281	**1.4 (1.0-1.8)**
Slug/snail baits	74/102	1.1 (0.7-1.6)
Rodenticides	59/67	1.3 (0.8-1.9)
Products for weeds	109/138	1.3 (0.9-1.9)
Indoor foggers for fleas	65/55	**1.5 (1.0-2.2)**

[1] The odds ratios are derived from conditional logistic regression, adjusted for household income; numbers in parentheses are 95% confidence intervals.

Pesticide Use: Dose-Response Relationship

Number of products	Cases/controls	OR[1]
None	51/86	1.0 --
1	74/95	1.5 (0.9-2.4)
2	72/98	1.5 (0.9-2.4)
3	64/82	2.0 (1.2-3.3)
4	62/53	3.1 (1.7-5.6)
5 or more	59/68	2.4 (1.4-4.2)
		P trend < 0.01

[1] OR=odds ratios adjusted for annual household income; Numbers in parentheses are 95% confidence intervals.

Similar dose-response relationships were observed separately for pesticide used during pregnancy and after birth, but not before conception

Pre- & Post-Natal Use of Indoor Insecticides

Exclusive or Combined Time Window of Exposure

Time window	Cases/controls	OR[1]
Never	53/97	1.0 --
Only before birth	29/17	2.9 (1.5-5.7)
Only after birth	86/115	1.3 (0.9-2.0)
Both before & after birth	185/216	1.7 (1.1-2.4)

[1] OR=odds ratios adjusted for annual household income; Numbers in parentheses are 95% confidence intervals.

Focus on Pre-Natal Use of Indoor Insecticides:
Exclusive or Combined Time Window of Exposure

Time window	Cases/controls	OR[1]
Never	159/231	1.0 --
Only before pregnancy	16/30	0.7 (0.4-1.4)
Only during pregnancy	78/65	1.9 (1.3-3.0)
Both before & during pregnancy	129/156	1.5 (1.1-2.1)

[1] OR=odds ratios adjusted for annual household income; Numbers in parentheses are 95% confidence intervals.

Conclusions

- Strong evidence that *in utero* and post-natal exposures to indoor insecticides are critical in the development of childhood leukemia.
  - No association is observed with preconception use
- Similar analyses conducted for outdoor herbicides showed increased risks with pre- and postnatal exposures
  - Numbers are limited to evaluate separate roles of preconception and *in utero* exposures.
- Associations mainly observed for ALL and Hispanic children, although differences by histologic type (ALL and AML) and ethnic group are not statistically significant with the current sample size.

Future Directions

- Refine assessment of environmental exposures.
  - Integrate other sources of pesticide exposure, such as drift or “take home” chemicals from outdoor areas and workplaces of parents.
  - Measure levels of selected pesticides in house dust and maternal urine samples.
  - Complete analyses on reliability of self-reports
- Identify genetic polymorphisms involved in the metabolism of pesticides.
  - E.g., PON1 gene polymorphism and organophosphate metabolism.
- Increase sample size.
  - Analyze by type of pesticide, by histologic and molecular subtype of leukemia, and by ethnic group.

NCCLS Strengths

- Large sample size – Expected total=1000 cases
- Comprehensive and detailed chemical exposure assessment
- Strong genetic and molecular components
- Research team able to evaluate environmental and genetic factors simultaneously

NCCLS Investigators

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NCCLS Collaborating Hospitals and Grants

- Children's Hospital Oakland
- Kaiser Permanente Medical Group in Oakland, San Francisco, Sacramento, Santa Clara
- UCSF School of Medicine
- Children's Hospital of Central California, Fresno
- Stanford University Lucille Packard Children's Hospital
- UC Davis School of Medicine

Childhood Leukemia (Grant: 2RO1ES09137-06)

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National Cancer Institute (Westat Contract # 015619)

Thank to the families participating in the NCCLS

NCCLS pesticide exposure methods

- Partial list of the 50 pesticides measured in carpet dust:

Lawn & garden Crop herbicides	Insecticides		Fungicides
2,4-D	Chlordane	Carbaryl	Ortho-phenylphenol
MCPA	DDE + DDT	Chlorpyrifos	
Dicamba	Dieldrin	Diazinon	
Trifluralin	Methoxychlor	Malathion	
Simazine	Heptachlor	Propoxur	