Birth Defects and Cancer:

The Intersection of Genes and Prenatal Exposures†

Atlanta, Georgia

September 10–11, 2012

WORKSHOP SUMMARY

†*This report was written by Drs. Somdat Mahabir and Martha Linet of the National Cancer Institute and members of the Workshop Steering Committee.*

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Workshop Background

Steering Committee Members

Somdat Mahabir, National Cancer Institute (NCI), National Institutes of Health (NIH)
Martha Linet, NCI, NIH
Andrew Olshan, University of North Carolina, Chapel Hill
Logan Spector, University of Minnesota
Gary Shaw, Stanford University
Jennita Reefhuis, Centers for Disease Control and Prevention (CDC)
Lorette Javois, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

Overview and Rationale

Research to understand how early-life events/factors affect cancer risk has been limited for several reasons, including methodological challenges. Childhood cancers are rare, and risk of developing a cancer before age 20 is approximately 3 per 1,000. Most pediatric cancers are sporadic, nonfamilial events. However, pediatric cancer may also occur in a child with a cancer-predisposing genetic condition (e.g., neurofibromatosis type 1, Li-Fraumeni syndrome, hereditary retinoblastoma).

Despite the high risks of cancer occurrence in children with such cancer-predisposing genetic conditions, not every child with these genetic conditions develops pediatric cancer. Childhood cancers may also occur in children who have chromosomal and nonchromosomal birth defects. Such co-occurrence may be due to underlying known genetic conditions, those not as yet identified, and possibly to unidentified environmental factors. This association between childhood cancer and birth defects may be due to gene-gene or gene-environment interactions. From the developmental biology perspective, the etiology of birth defects and cancer shares a common nexus in the genetic pathways that govern signal transduction and cell growth. Epidemiologic and genetic investigations of this association may provide new insights into the etiology of these diseases and certain adult-onset cancers.

Recent developments from epidemiologic, genetic, and experimental studies provide new impetus for concerted efforts to examine the intersection of periconceptional and prenatal teratogenic and carcinogenic exposures, lifestyle factors, and genes.

Purpose

The objective of the Workshop was to bring together experts/scientists conducting research on birth defects and cancer to address the intersection of childhood cancer and birth defects, including the influence of prenatal exposures and genetic factors. The Workshop brought together a multidisciplinary group of experts to assess progress made and prioritize research gaps. The scientists were experts in the epidemiology of childhood cancers and birth defects,

genomics and genetic syndromes, developmental pathways, animal model systems, registries, other relevant databases, and mathematical model systems.

In this Workshop, experts presented their perspectives on major facets of birth defects and cancer research, focusing on the intersection of genes and prenatal, periconceptional, or preconception exposures that may predispose to occurrence of:

1. Birth defects,
2. Pediatric cancers arising in children with birth defects, and
3. Pediatric cancer-predisposing genetic conditions:
    - Chromosomal genetic syndromes, and
    - Nonchromosomal genetic syndromes

This Workshop focusing on **"Birth Defects and Cancer Risk: The Intersection of Genes and Prenatal Exposures"** was a first step in highlighting opportunities and challenges in improving and developing new methodologies and resources to study the role of genetics, environmental exposures, and gene-environment interactions in etiologies of childhood cancers and birth defects. For each topic, experts with diverse perspectives offered an assessment of the state-of-the-science, major challenges, and most-attractive research opportunities. Moderators led discussions between the speakers and participants.

There was a panel discussion at the end of the Workshop to identify key research gaps and prioritize major recommendations to enhance the nexus between birth defects and cancer, with the ultimate goal of preventing these conditions.

Deliverables and Outcomes

1. Workshop report.
2. Project ideas that could be developed into NCI applications (potentially with accompanying Requests for Applications (Nöthlings, Schulze et al. 2008) or Program Announcements [PA]). Such Funding Opportunity Announcements could include mechanisms to link national registries and systems; leverage ongoing and past case-control, other epidemiologic studies, and/or genomic studies; and encourage interdisciplinary collaborations to further progress.
3. Opportunities to initiate linkage between birth defects, exposure data (prenatal, preconception, environmental factors) and childhood cancers.
4. Opportunities to create an infrastructure for the linking and pooling of data for research purposes.

The Workshop Agenda follows.

Agenda

September 10, 2012

Time	Agenda Item
8:30 a.m. – 9:00 a.m.	**Registration**
9:00 a.m. – 9:15 a.m.	**Introductory Remarks**
9:15 a.m. – 9:30 a.m.	**Workshop Overview and Objectives** **Speaker:** Somdat Mahabir, Ph.D., M.P.H. *Program Director, Environmental Epidemiology Branch* *Epidemiology and Genomics Research Program* *Division of Cancer Control and Population Sciences* *National Cancer Institute (NCI)*
9:30 a.m. – 10:15 a.m.	**Epidemiology of Birth Defects and Cancer: State of the Evidence, Opportunities, and Challenges** **Keynote Speaker:** Andrew Olshan, Ph.D. *Professor and Chair, Department of Epidemiology* *Gillings School of Global Public Health* *University of North Carolina, Chapel Hill*
10:15 a.m. – 10:30 a.m.	**Discussion Moderator:** Jennita Reefhuis, Ph.D. *Centers for Disease Control and Prevention (CDC)*
10:30 a.m. – 10:45 a.m.	**Break**
10:45 a.m. – 11:15 a.m.	**Genomic Causes in Birth Defects—Cancer Nexus** **Speaker:** Katherine A. Rauen, M.D., Ph.D. *Associate Professor* *Director, NF/Ras Pathway Clinic* *Associate Director, Medical Genetics Residency Program* *UCSF Helen Diller Family Comprehensive Cancer Center*
11:15 a.m. – 11:30 a.m.	**Discussion Moderator:** Logan Spector, Ph.D. *University of Minnesota*
11:30 a.m. – 12:00 p.m.	**Developmental Pathways Linking Birth Defects to Cancer** **Speaker:** Alexandra L. Joyner, Ph.D. *Courtney Steel Chair in Pediatric Cancer Research* *Memorial Sloan-Kettering Cancer Center*
12:00 p.m. – 12:15 p.m.	**Discussion Moderator:** Lorette Javois, Ph.D. *Eunice Kennedy Schriver National Institute of Child Health and Human Development (NICHD)*
12:15 p.m. – 1:00 p.m.	**Lunch**

September 10, 2012 (continued)

Time	Agenda Item
1:00 p.m. – 1:30 p.m.	**Experimental Insights Into the Mechanisms Linking Birth Defects to Cancer** **Speaker:** Lucy Anderson, Ph.D., D.A.B.T. *Chief, Cellular Pathogenesis Section (Retired)* *Laboratory of Comparative Carcinogenesis, NCI*
1:30 p.m. – 1:45 p.m.	**Discussion Moderator:** Gary Shaw, Dr.P.H. *Stanford University*
1:45 p.m. – 2:15 p.m.	**Methodological Challenges in Studying Birth Defects and Cancer** **Speaker:** W. Dana Flanders, M.D., D.Sc. *Professor, Department of Epidemiology, Biostatistics, and Bioinformatics* *Rollins School of Public Health, Emory University*
2:15 p.m. – 2:30 p.m.	**Discussion Moderator:** Somdat Mahabir, Ph.D., M.P.H. *NCI*
2:45 p.m. – 3:15 p.m.	**Opportunities to Link Databases to Study Birth Defects and Subsequent Cancer Development** **Speaker:** Martha Linet, M.D., M.P.H. *Chief and Senior Investigator, Radiation Epidemiology Branch* *Division of Cancer Epidemiology and Genetics, NCI*
3:15 p.m. – 3:30 p.m.	**Discussion Moderator:** Andrew Olshan, Ph.D. *University of North Carolina, Chapel Hill*
3:30 p.m. – 3:45 p.m.	**What Do We Know About the Role of Birth Defects and Prenatal Exposures as Risk Factors for Childhood Cancer, and What Are the Future Recommendations for Research?** **Remarks by Moderator:** Gary Shaw, Dr.P.H. *Professor, Pediatrics-Neonatal and Developmental Medicine* *Associate Chair, Clinical Research* *Stanford University School of Medicine*
3:45 p.m. – 4:30 p.m.	**Panel Discussion and Short Presentations** Martha Linet, M.D., M.P.H., *NCI* Andy Olshan, Ph.D., *University of North Carolina, Chapel Hill* Sonja A. Rasmussen, M.D., M.S., *CDC* Julie Ross, Ph.D., *University of Minnesota* Sharon Savage, M.D., F.A.A.P., *NCI* Logan Spector, Ph.D., *University of Minnesota*
4:30 p.m. – 5:15 p.m.	**Questions for Panelists**
5:15 p.m. – 5:30 p.m.	**Summary and Follow-Up** Somdat Mahabir, Ph.D., M.P.H. *NCI*
5:30 p.m.	Adjournment

September 11, 2012

Time	Agenda Item
9:00 a.m. – 1:00 p.m.	**Steering Committee Members, Speakers, Moderators (closed deliberations)** Summary of the Workshop—Scope of the Problem, Gaps, Future Directions Specific Recommendations for Actions Workshop Follow-Up Report

Summary Report—Workshop on Birth Defects and Cancer: The Intersection of Genes and Prenatal Exposures

Introduction and Background

What Is the Intersection Between Birth Defects and Cancer?

Children with certain types of birth defects are at increased risk of developing specific types of childhood cancer (**Table 1**). The first report describing the occurrence of acute lymphoblastic leukemia in a child with Down syndrome was published in 1930 (Brewster and Cannon 1930). Since then, there have been numerous confirmatory clinical reports, a detailed assessment of morphological abnormalities in a large series of childhood cancer patients (Merks, Ozgen et al. 2008), registry-based studies (Mili, Khoury et al. 1993, Bjørge, Cnattingius et al. 2008, Fisher, Reynolds et al. 2012), and a limited number of analytical investigations (Canfield, Spector et al. 2004).

Along with improvements in the classification of birth defects and childhood cancer and the establishment of large and increasingly long-standing population-based surveillance systems for birth defects and cancer registries, notable advances have been made in understanding key developmental pathways that control proliferation and differentiation. Recognition of ways in which molecular and genetic alterations in these pathways might result in birth defects, pediatric cancer, or both also has increased. An enhanced understanding of the structural and functional aspects of the genes that underpin genetic syndromes has led to recognition that germline mutations and somatic dysregulation of key genes in important pathways may support the occurrence of genetic syndromes that can predispose to increased cancer occurrence in children or adult family members. Although teratogenesis and perinatal carcinogenesis were long thought to arise from different mechanisms in animal models, new knowledge suggests new mechanisms of action by which a common exposure may cause both birth defects and cancers. More recent large-scale, high-quality, registry-based, and analytic epidemiologic studies, together with findings from genomics investigations, provide new clues that could be pursued in studies of carcinogenesis among children with birth defects and their close family members.

Why Study the Intersection Between Birth Defects and Pediatric Cancer?

Birth defects and childhood cancers are leading causes of infant and childhood morbidity and mortality. Birth defects account for more than 20 percent of all infant deaths (http://www.cdc.gov/ncbddd/birthdefects/data.html), and cancer is the second leading cause of death (after accidents) in children younger than 15 years old (http://www.cancer.org/cancer/cancerinchildren/detailedguide/cancer-in-children-key-statistics). Data from U.S. birth defects surveillance programs reveal that most of the specific major birth defects occur in fewer than 50 per 100,000 live births (Parker, Mai et al. 2010). Population-based cancer registries have reported that recent age-adjusted incidence of the 12 major categories of childhood cancer ranges from 0.05 to 5.2 per 100,000 per year among children ages 0–14 years old (Howlader, Noone et al. 2013).

Birth defects and childhood cancers each comprise heterogeneous entities that are defined by phenotypic, genetic, molecular, and clinical characteristics. These features are increasingly incorporated into evolving classification systems for birth defects (Rasmussen, Olney et al. 2003) and childhood cancers (Steliarova-Foucher, Stiller et al. 2005).

Many descriptive and analytical epidemiologic studies have been undertaken following reports from seminal studies that linked exposure to the rubella virus and thalidomide during pregnancy with specific birth defects (Gregg 1941) and diagnostic x-rays during pregnancy with childhood leukemia and other pediatric cancers (Stewart, Webb et al. 1958; MacMahon 1962). Descriptive studies of birth defects have been hampered by the difficulty of achieving complete ascertainment of birth defects due to fetal loss, and failure to identify subtle structural defects and those characterized by primarily functional characteristics. A comparison of the prevalence of birth defects across populations demonstrated quantifiable variation (WHO 2003), similar to the international variation described in childhood cancers (Stiller and Parkin 1996). It has been estimated that approximately 15 percent of birth defects can be attributed to single-gene disorders (10 percent) and chromosomal syndromes (5 percent) (Brent 2004), and 4–5 percent of childhood cancers have been attributed to genetic syndromes (Narod, Stiller et al. 1991). Although these estimates suggest that environmental agents and, to a greater extent, gene-environment interactions, account for the majority of these disorders, few risk factors have been consistently confirmed for specific types of birth defects and childhood cancers, and the etiology of most of these entities remains unexplained (Khoury, Becerra et al. 1989; Gilbert 2010; Olshan AF 2011).

What Has Been Learned to Date About the Relationship Between Birth Defects and Cancer?

Clinical and Epidemiologic Studies

Childhood cancers and birth defects are rare conditions with largely unknown etiologies. Most of the specific major birth defects occur in fewer than 5 per 10,000 live births (Parker, Mai et al. 2010). To recruit sufficient numbers of cases for epidemiologic investigations, etiologic studies on childhood cancers or birth defects typically are conducted as large population-based case-control studies. Given the rare co-occurrence of these outcomes in the general population, obtaining adequate statistical power in epidemiologic studies investigating the links between childhood cancers and birth defects presents a challenge.

Evidence of a connection between childhood cancers and birth defects comes from three major sources: clinical observations of syndromes, registry linkages, and case-control studies. Registry linkages typically yield the highest number of joint cases but usually provide less information on potential risk factors than case-control studies. More than 10 registry linkage studies have been published in United States and Europe (Mili, Khoury et al. 1993, Mili, Lynch et al. 1993, Hill, Gridley et al. 2003, Agha, Williams et al. 2005, Bjørge, Cnattingius et al. 2008, Rankin, Silf et al. 2008, Sípek, Malis et al. 2009, Carozza, Langlois et al. 2012, Fisher, Reynolds et al. 2012). The largest study, conducted in Sweden and Norway, included more than 120,000 cases of birth defects among 5 million births and observed 622 comorbid cases of birth defects and cancer (Bjørge, Cnattingius et al. 2008). In the United States, two large registry linkages were

conducted recently in California and Texas, each with more than 3 million births (Carozza, Langlois et al. 2012, Fisher, Reynolds et al. 2012).

Children with certain types of birth defects are at increased risk of developing specific types of childhood cancer (**Table 1**). As noted above, the first report describing the occurrence of acute lymphoblastic leukemia in a child with Down syndrome was published in 1930 (Brewster and Cannon 1930). Subsequent publications include numerous confirmatory clinical reports, a detailed assessment of morphological abnormalities in a large series of childhood cancer patients (Merks, Ozgen et al. 2008), registry-based studies (Mili, Khoury et al. 1993, Mili, Lynch et al. 1993, Bjørge, Cnattingius et al. 2008, Fisher, Reynolds et al. 2012), and a limited number of analytical investigations (Canfield, Spector et al. 2004).

Collectively, these studies demonstrate that children with a variety of birth defects have a significantly increased risk of developing several types of childhood cancers. The risk is highest among children with chromosomal defects, although studies that exclude these anomalies also report increased risk among infants with major nonchromosomal structural defects or minor morphological abnormalities. Most studies do not have sufficient size to evaluate the potential relation between individual birth defects and cancer phenotypes. However, some studies consistently report associations between all defects and specific cancers, or specific defects and all cancers. For example, in the California and Texas studies, the risk of developing childhood cancer was approximately 2- to 4-fold higher among infants with defects of the respiratory, genitourinary, and central nervous systems, as well as congenital heart defects. The established association between Down syndrome and childhood leukemia is strongest in magnitude; children with Down syndrome are more than 10 times more likely to develop acute lymphoblastic or myeloid leukemia than those without Down syndrome. Among children with birth defects, the risk of developing childhood cancer appears to increase with the number of diagnosed anomalies. The risk of childhood cancer among those with birth defects is highest during infancy and declines with increasing age.

Overlapping Risk Factors for Birth Defects and Childhood Cancer

Assessing risk factors associated with cancer in children with specific types of birth defects may be facilitated by examining characteristics and exposures that evidence links to both birth defects and childhood cancer. As shown in **Table 2**, there appears to be some overlap in certain demographic, socioeconomic, lifestyle, dietary, medical, reproductive, infectious, environmental, and parental occupational agents associated with risk of specific birth defects and specific cancers. Inconsistency in the associations observed among epidemiologic studies must be acknowledged, however. These inconsistencies may reflect problems in measurement (because the source of data for most exposures is maternal recall), recall, or other forms of bias (because mothers of healthy control children may not remember exposures during pregnancy in the same way as mothers of children with birth defects or cancer), or may reflect different proportions of susceptible subgroups in the study population.

Reports to date are limited for some particularly promising areas for further epidemiologic research, but there is potential for prevention or intervention. These areas include clarification of whether racial/ethnic differences represent differences in modifiable exposures, and the role of

paternal pre- or periconceptional smoking; assisted reproductive technologies; gestational maternal diabetes; prepregnancy obesity; and maternal environmental, parental occupational, and maternal dietary exposures. With larger future studies, specific birth defect-cancer associations can be refined, and potential shared risk factors can be more thoroughly evaluated, including nutritional status and prenatal vitamin supplementation, maternal and paternal smoking and alcohol use, maternal infertility and assisted reproductive technologies, radiation exposure, environmental and occupational exposures, and genetic factors.

Embryogenesis and Important Pathways

During embryogenesis, an evolutionarily conserved, fairly small set of paracrine factors is used repeatedly across multiple organ systems to induce organ formation. These factors have been grouped into four major families: fibroblast growth factors, hedgehog (HH) family, Wnt family, and transforming growth factor-beta (TGF-β) family. All are responsible for initiating signal transduction cascades. The major signal transduction cascades are molecularly complex, and all are variations on a common theme. Their ultimate endpoints regulate either gene expression or cytoskeletal changes, thus causing cell proliferation, specification, or differentiation, including migration (Gilbert 2010). It is not surprising that alterations of these fundamental developmental pathways that control proliferation and differentiation would lead not only to birth defects but also to the onset and progression of cancer. Indeed, as the molecular details of these pathways have been revealed over the years, researchers who study development and cancer have found themselves studying the same genes.

Relationship of Teratogenesis and Perinatal Carcinogenesis

In animal models, most strongly genotoxic transplacental carcinogens also are teratogens. Extensive historical animal studies noted a lack of temporal overlap for sensitivity to teratogenesis vs. transplacental carcinogenesis by genotoxicants, however. Teratogenesis commonly occurs after groups of cells are exposed during organogenesis during the first third of gestation; whereas carcinogenic effects usually are detectable only from treatment during the last third of gestation, after tissue differentiation, with single cells as the target. Furthermore, numerous nongenotoxic agents and conditions are teratogenic but not carcinogenic in both animals and humans. These observations led to the conclusion that the pathological processes of teratogenesis and fetal carcinogenesis are mechanistically distinct and unrelated (Alexandrov 1973).

More recent investigations in animals have strongly implicated reactive oxygen species (ROS) in the mechanism of action of some teratogenic agents (Wells, McCallum et al. 2009). Investigators have suggested that ROS also are involved in the transplacental carcinogenic effects of genotoxicants (Wan and Winn 2006, Wells, McCallum et al. 2009). If this is true, then the same ROS-generating agent or condition, experienced repeatedly or chronically over the course of gestation, could result in both birth defects and postnatal cancers. At present there is little direct experimental evidence to support this important idea, but it could be fruitfully tested in genetically engineered mouse models with deficiencies in antioxidant or DNA repair proteins.

A related possibility is that an exposure causes both birth defects and neoplasms through a two-step process. In the first step, the exposure might alter undifferentiated tissue such that a physical

or functional defect results and, at the same time, alter cellular programming in a way that increases the likelihood of later initiation of a neoplasm. This idea also could be modeled in animals. As an example, in humans, neural tube birth defects are associated with both maternal diabetes and central nervous system cancers (Bjørge, Cnattingius et al. 2008, Zabini S 2010). A mouse model for induction of neural tube defects by embryo exposure to high glucose on gestation day 7.5 (Fine, Horal et al. 1999) could be combined with a genetic mouse model for brain tumors caused by chemicals or radiation at the end of gestation (Takahashi, Matsuo et al. 2012). Paternal exposures also could be tested for two-step effects, especially for functional teratogenesis.

Genetic Studies in Humans

Insights from RASopathies

The RASopathies are a clinically defined group of genetic syndromes that are caused by germline mutations in genes that encode components or regulators of the Ras/mitogen-activated protein kinase (MAPK) pathway (Tidyman and Rauen 2009). The Ras/MAPK pathway has been studied extensively in the context of oncogenesis because its somatic dysregulation is a primary cause of cancer. RAS has been found to be somatically mutated in approximately 20 percent of malignancies (Bos 1989), and BRAF is somatically mutated in approximately 7 percent of malignancies [for a review, see [Pritchard, 2007 #14]]. In addition, the Ras/MAPK pathway plays a vital role in development.

The RASopathies represent the quintessential model for the intersection of cancer and birth defects. Each RASopathy exhibits unique phenotypes due to a common pathogenic mechanism that results in Ras/MAPK pathway dysregulation; however, RASopathies share many overlapping characteristics. These include craniofacial dysmorphology; cardiac malformations; cutaneous, musculoskeletal, and ocular abnormalities; neurocognitive impairment; hypotonia; and increased cancer risk. Taken together, the RASopathies are one of the largest groups of malformation syndromes known, affecting approximately 1 in 1,000 individuals. Neurofibromatosis type 1 (NF1) was the first syndrome to be identified as being caused by germline mutations in the Ras/MAPK pathway (Cawthon, O'Connell et al. 1990, Viskochil, Buchberg et al. 1990, Wallace, Marchuk et al. 1990). Subsequently, numerous other syndromes have been identified as well. These syndromes include: 1) Noonan syndrome (NS), caused by activating mutations in *PTPN11* (Tartaglia, Mehler et al. 2001), *SOS1* (Tartaglia, Mehler et al. 2001, Roberts, Araki et al. 2007), *RAF1* (Pandit, Sarkozy et al. 2007, Razzaque, Nishizawa et al. 2007), *KRAS* (Schubbert, Zenker et al. 2006), *NRAS* (Cirstea, Kutsche et al. 2010), *SHOC2* (Cordeddu, Di Schiavi et al. 2009), and *CBL* (Martinelli, De Luca et al. 2010, Niemeyer, Kang et al. 2010); 2) NS with multiple lentigines, caused by mutations in *PTPN11* (Digilio, Conti et al. 2002) and *RAF1* (Pandit, Sarkozy et al. 2007); 3) capillary malformation-arteriovenous (AV) malformation, caused by haploinsufficiency of *RASA1* (p120-RasGAP) (Eerola, Boon et al. 2003); 4) Costello syndrome (CS), caused by activating mutations in *HRAS* (Aoki, Niihori et al. 2005); 5) cardiofaciocutaneous syndrome (CFC), caused by alterations in MAPK pathway activation by activating mutations in *BRAF* (Niihori, Aoki et al. 2006, Rodriguez-Viciana, Tetsu et al. 2006) and *MAP2K1/MEK1* or *MAP2K2/MEK2* (Rodriguez-Viciana, Tetsu et al. 2006); and 6) Legius syndrome, caused by inactivating mutations in the *SPRED1* gene (Brems, Chmara et al. 2007). The RASopathies share a predisposition for increased risk of certain malignancies,

including juvenile myelomonocytic leukemia, rhabdomyosarcoma, and acute lymphoblastic leukemia, but it remains unclear why some syndromes and individuals are at increased risk for cancer risk while others are not.

Other examples

The HH pathway regulates development of all organs and is extremely important in limb development, neural patterning, and craniofacial morphogenesis. Increasing evidence shows that the HH pathway also regulates adult stem cells in diverse organs (Varjosalo and Taipale 2008). Mutations that deactivate this signaling pathway cause malformations, including holoprosencephaly. Alternatively, mutations that activate the pathway ectopically cause cancers, as seen in Gorlin syndrome (medulloblastomas). Gorlin syndrome, also called basal cell nevus syndrome, is a rare condition in which mutation of the HH receptor, patched, causes both structural anomalies (fused fingers and rib and facial defects) and multiple malignant tumors—including medulloblastomas, the most prevalent childhood malignant tumor, and basal cell carcinoma, the most common cancer in the United States (Barakat, Humke et al. 2010, Low and de Sauvage 2010).

Gaps in Knowledge

Little is known about the determinants of carcinogenesis in persons with birth defects. Recent epidemiologic studies primarily have been descriptive in nature (e.g., linked registry studies); analytical studies are needed that focus on the role of environmental exposures, genetic characteristics, and gene-environment interaction. Comprehensive understanding of genetics and mechanisms is lacking. Mouse models and genetic mouse models could be utilized to improve understanding of teratogenesis and the role and timing of potential carcinogenic exposures in genetically modified animals.

Methodological Challenges

Methodological challenges must be addressed in the design and implementation of new epidemiologic, genetic, and experimental studies. Among the challenges are the: 1) rarity of birth defects and cancer occurrence in those with birth defects; 2) limitations of birth defects classification systems and need for expertise in clinical genetics; 3) evolving classification of pediatric cancers; 4) need for optimal and new study designs for studying the intersection of birth defects and cancer; 5) difficulties of assessing prenatal and preconceptional exposures retrospectively; 6) rapid evolution of genomics and need to develop high-quality study designs to assess genomics in persons with birth defects; 7) need to identify methods and incentives to encourage participation and retention of persons with birth defects in longitudinal studies; and 8) unique confidentiality and privacy sensitivities of studies that involve persons with birth defects and special needs.

Approaches for Future Studies

Workshop recommendations regarding future studies included:

1. Identify the most pressing hypotheses to pursue;

2. Determine optimal study designs and develop new study designs to address these hypotheses;

3. Capitalize on existing databases/studies; and

4. Establish a working group to move forward.

Although certain forms of birth defects have consistently been associated with childhood cancer and numerous examples of known gene defects underlie both phenotypic events, large gaps in our etiologic knowledge persist. As discussed above, registry studies have linked combined birth defects to specific childhood cancers and specific birth defects to combined childhood cancers, but the studies have not reached sufficient size to achieve specificity for both conditions. Aggregation of data from registry studies is hampered by the lack of systematic and standardized methods in the ascertainment and classification systems used in different studies that have reported results from registry linkage of birth defect and cancer incidence data. Fortunately, a single standard system is used to classify childhood cancers (Steliarova-Foucher, Stiller et al. 2005).

Whether cancers in children with birth defects display histologic, cytogenetic, or mutational differences compared with the same types of cancers in children without birth defects is unknown but may shed light on the timing of the co-development of each condition. For instance, although many leukemic translocations have been shown to occur *in utero*, some, such as E2A-PBX1, appear to be acquired postnatally (Wiemels, Leonard et al. 2002). The association of birth defects with E2A-PBX1 would imply a chronic exposure or genetic defect, rather than a transient exposure early in gestation. Birth defects are not always recorded at diagnosis of cancer. It should be possible to put in place a systematic assessment of birth defects in newly diagnosed cancer cases at enough large centers to rapidly accrue a sufficient number of patients. Others have demonstrated the power of applying the systematic assessment of dysmorphology to a series of patients over many years (Merks, Ozgen et al. 2008). In the long-term, it would be helpful to encourage the addition of dysmorphologic examinations and birth defect histories to the standard patient history used by pediatric oncologists.

Next-generation sequencing is increasingly used to identify variants underlying clearly Mendelian disorders (Swami 2010) and those with more complex inheritance (Eichler, Flint et al. 2010). These investigations typically are premised on having multiple family members with the same or similar conditions. It may be productive, however, to consider families with a combination of childhood cancers and birth defects as candidates for sequencing.

Sequencing patients with both birth defects and childhood cancer, even absent a suggestive family history, may be productive. In the presence of other phenotypic abnormalities, co-occurrence of phenotypes may signify either gene deletions or homozygous recessive mutations, which are relatively easy to identify. However, incompletely penetrant variants will be almost impossible to associate with a trait in individual patients.

The role of somatic mutation also should be considered. All cancers are thought to arise from somatically mutated cells that must take root in a permissive microenvironment (Bissell and Hines 2011). Somatic mosaicism recently has been demonstrated in the general population at a surprisingly high frequency (Rodriguez-Santiago, Malats et al. 2010); almost 2 percent of

subjects displayed mosaicism in a large enough fraction of lymphocytes to be detectable on genomewide single nucleotide polymorphism (SNP) arrays. Moreover, lymphocyte mosaicism recurs in areas of the genome that frequently are altered in hematopoietic cancers and is strongly associated with their risk (Jacobs, Yeager et al. 2012). The true incidence of mosaicism will require a wider sampling of tissues, some of which will be difficult to examine except postmortem. It may be of interest, however, to examine mosaicism in tissue collected during corrective surgery for birth defects.

The following were cited among the considerations for going forward:

- The National Birth Defects Prevention Study (NBDPS) and the epidemiologic working group of the Children's Oncology Group are two relevant U.S. consortia that could join forces to further explore these areas.

- A small working group of members of the NBDPS and the Children's Oncology Group (COG), along with representatives from the NCI, NICHD, and CDC, could be established to identify promising hypotheses such as those listed in **Table 2** and approaches to pursue, including types of study designs.

Table 1. Childhood cancers associated with birth defects and inherited syndromes

Type of childhood cancer **Type of birth defect or inherited syndrome**	**Acute lympho-cytic leukemia**	**Acute myeloid leukemia**	**Non-Hodgkin lymphoma**	**Central nervous system tumors**	**Retino-blastoma**	**Renal/ Wilms' tumor**	**Hepato-blastoma**	**Bone tumors/ osteo-sarcomas**	**Soft tissue sarcomas**
Down syndrome	X	X							
Neuro-fibroma-tosis	X	X		X					X
Langerhans cell histio-cytosis	X								
Klinefelter syndrome	X								
Familial monosomy 7		X							
Kostmann granulo-cytopenia		X							
Fanconi anemia		X							
Schwach-man syndrome	X	X							
Bloom syndrome	X	X							
Ataxia telangiec-tasia	X		X						
Wiskott-Aldrich syndrome			X						

Type of childhood cancer **Type of birth defect or inherited syndrome**	**Acute lympho-cytic leukemia**	**Acute myeloid leukemia**	**Non-Hodgkin lymphoma**	**Central nervous system tumors**	**Retino-blastoma**	**Renal/ Wilms' tumor**	**Hepato-blastoma**	**Bone tumors/ osteo-sarcomas**	**Soft tissue sarcomas**
X-linked lymphopro-liferative disease			X						
Noonan syndrome		X Juvenile myelo-monocytic leukemia							X
Costello syndrome									X
Cardio-facio-cutaneous syndrome	X	X Juvenile myelo-monocytic leukemia	X				X		
Li-Fraumeni syndrome				X				X	X
Tuberous sclerosis				X					
Nevoid basal cell syndrome				X					
Turcot's syndrome				X					
Familial retino-blastoma/ 13q deletion					X				
Aniridia						X			
WAGR syndrome						X			
Perlman syndrome						X			

Type of childhood cancer **Type of birth defect or inherited syndrome**	**Acute lympho-cytic leukemia**	**Acute myeloid leukemia**	**Non-Hodgkin lymphoma**	**Central nervous system tumors**	**Retino-blastoma**	**Renal/ Wilms' tumor**	**Hepato-blastoma**	**Bone tumors/ osteo-sarcomas**	**Soft tissue sarcomas**
Denys-Drash syndrome						X			
Simpson-Golabi-Behmel syndrome						X			
Beckwith-Wiede-mann syndrome						X	X		X
Familial adenoma-tous polyposis							X		
Gardner syndrome							X		
Hemi-hyper-trophy						X	X		
Rothmund-Thomson syndrome								X	

Table 2. Risk factors linked with both specific birth defects and specific childhood cancers

Characteristics or agents	**Linked with specific birth defects Strength of evidence (none, +, ++, +++)** **Factor linked with which defects**	**Linked with specific childhood cancers Strength of evidence (none, +, ++, +++) Factor linked with which cancer**
Sex	**+++** Most birth defects are characterized by higher rates in males than in females	+++ Most childhood cancers are characterized by higher rates in males than in females, except Wilms' tumor
Parental age	+++ Down syndrome (maternal)	++ leukemia (paternal) +-++ leukemia, lymphoma, brain, neuroblastoma, Wilms', bone tumors, soft tissue sarcomas (maternal)
Race/Ethnicity	++ Anencephalus, spina bifida (Hispanics) + conotruncal, dextrotransposition, coarctation of aorta (whites) + atrial and ventricular septal defects (Hispanics)	+++ Ewing's sarcoma, acute lymphoblastic leukemia
Socioeconomic status	++ neural tube defects	+ leukemia, Hodgkin lymphoma
Parental smoking	++ congenital heart defects	++ leukemia (paternal pre- and peri-conceptional smoking)
Maternal alcohol use	+++ fetal alcohol syndrome	+ acute myeloid leukemia
Maternal use of specific drugs	+++ Diethylstilbestrol (urovaginal malformations, testicular anomalies), thalidomide (limb, ear, heart defects), diphenylhydantoin (heart, craniofacial, microcephaly), trimethadione (ear, palate, heart), warfarin (microcephaly, chondrodysplasia), iodides (mental retardation, goiter), valproic acid (spina bifida), isotretinoin (many)	+++ Diethylstilbestrol (vaginal adenocarcinoma; breast cancer)
Assisted reproductive technology	++ cardiac defects, esophageal atresia, anal atresia, hypospadias	
Preterm births	++ central nervous system (CNS), cardiovascular	
Maternal diabetes mellitus	++ CNS defects, limb deficiencies, renal agenesis, hypospadias, orofacial clefts, heart defects, multiple defects	
Maternal prepregnancy obesity	++ spina bifida + heart defects, anorectal atresia, hypospadias, limb reduction defects, diaphragmatic hernia, omphalocele	

Characteristics or agents	**Linked with specific birth defects Strength of evidence (none, +, ++, +++) Factor linked with which defects**	**Linked with specific childhood cancers Strength of evidence (none, +, ++, +++) Factor linked with which cancer**
Other maternal diseases	++ virilizing tumors (masculinization of female offspring); phenylketonuria (microcephaly, growth retardation, heart defects)	
Maternal infectious diseases	+++ rubella (cardiovascular and other defects, deafness); cytomegalovirus (CNS and eye defects); herpes simplex (CNS defects); varicella (limb atrophy, low birth weight, eye defects)	
Maternal exposure to radiation		++ diagnostic radiation to the abdomen or pelvis (leukemia)
Maternal occupational exposures	+ volatile organic solvents (oral clefts), glycol ethers (neural tube defects, oral clefts), some pesticides (neural tube defects, limb defects), lead (neural tube defects)	
Paternal occupational exposures	+ janitors, painters, printers, occupations with exposure to solvents, firefighters, agricultural occupations (various birth defects)	
Dietary	+++ folic acid supplements (neural tube defects) ++ dietary folate (anencephaly) ++ folate-related micronutrient involved in one-carbon metabolism (neural tube defects, anencephaly)	+ folic acid supplements (leukemia, brain) + dietary folate (leukemia, brain) + cured meats

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