Process for approval of genetic studies

Application of Intent

Concept Development

Concept Review

(Working group chair, statistician)

- **Concept to include**
  - Hypothesis, aims
  - Background and rationale
  - Study design
  - Nature and quantity of specimens
  - Sample size and power
  - Funding source
  - Biosketch of PI

Prioritization for approval of concept

- Specimen availability
- Scientific merit
- Technical merit
- Extramural funding

Concept Review

(Publications Committee)

Published Studies

Concept # 1   GWAS of SMNs after Hodgkin lymphoma

PI   **Ken Onel, U of Chicago**

GWAS of SMNs after Hodgkin lymphoma

Aims   GWAS of SMNs in patients treated for HL with RT

Design   Matched case-control study design

- Discovery set
    - 103 cases with SMNs and 121 controls with no SMNs
- Replication set
    - 120 cases and 112 controls

Funding   **NIH (R21)**

Results   Two variants at chromosome 6q21 associated with SMN

- Variants associated with decreased basal PRDM1 expression and impaired induction of *PRDM1* by radiation exposure
- Implicates *PRDM1* in the etiology of RT-induced SMNs
    - *PRDM1* involved in proliferation, differentiation, apoptosis

Best T et al, **Nat Med** *2011;17:941-3*

Concept # 2   **Evaluation of SNPs in the EWS Breakpoint Region in Individuals with/ without Ewing sarcoma**

PI   Stephen DuBois, UCSF

Evaluation of SNPs in the EWS Breakpoint Region in Individuals with and without Ewing sarcoma

Primary Aim
Determine if frequency of SNPs in the *EWSR1* gene differs between individuals with and without Ewing sarcoma
18 tagged HapMap SNPs that span entire *EWSR1* gene

Secondary Aims
Compare frequency of SNPs in the *EWSR1* gene between patients with Ewing sarcoma who survived 5 years from initial diagnosis and those who did not

Determine frequency of any SNPs associated with Ewing sarcoma in a control population of African ancestry

Results
Variations in *EWSR1* at known SNPs or across intron 7 are not associated with diagnosis of Ewing sarcoma

EWSR1 does not appear to be an Ewing sarcoma susceptibility gene

Dubois S et al. Pediatr Blood Cancer, 2011

Concept # 3   **Genomic alterations in radiation-related breast cancer using Array-CGH (Comparative Genomic Hybridization)**

PI   Rose Yang, NCI

Genomic alterations in radiation-related breast cancer using Array-CGH

Aims Using array-CGH, estimate prevalence/ patterns of DNA copy number changes in radiation-exposed breast tumor tissues

- Identify distinct genomic aberrations related to age at exposure and type of radiation (acute vs. protracted)
  – Compare CGH profiles among breast tumors exposed to high-dose (CCSS), low-dose (Lifespan), and no XRT(data)

Results Array-CGH data obtained from 25 CCSS cases

- Invasive ductal tumors (n=19); ER-positive staining (15 of 22),
- High proliferation (high Ki-67 staining – 17 of 21)
- Pattern of copy number changes similar to sporadic breast ca

Status Radiat Res (major revision)

Criticism – lack of comparison with breast cancer without radiation

Response – conduct studies in age- and hormone-receptor-matched cohort of de novo breast cancer

Concept # 4   Telomere length and Second Malignancy in Pediatric Cancer Survivors

PI   Monica Gramatges, Baylor

Telomere length and Second Malignancy in Pediatric Cancer Survivors

Hypothesis   Shortened germline telomere length plays a role in development of SMNs in childhood cancer survivors

- increased likelihood for mutational gains/losses in an already strained checkpoint system upon genotoxic exposure

Aims   Investigate relation between telomere length in buccal DNA samples and SMNs in childhood cancer survivors

Methods   Matched case-control study design

cases: n=147; controls: n=147

Breast ca (n=68); thyroid ca (n=48); sarcoma (n=31)

qPCR analysis to measure telomere length

Results   Preliminary findings support hypothesis for thyroid cancer

Plans   Plan to submit to Clin Cancer Res

Concept # 5   Genetic Alterations in Second Malignant Neoplasms

PI   Jean Nakamura, UCSF

Genetic Alterations in Second Malignant Neoplasms

Aim 1   Determine whether LOH in tumor suppressor genes (previously identified in the *Nf1* mutant mouse model) occurs in SMNs

SNP genotyping (Taqman) on DNA isolated from FFPE SMN samples

Initial analysis to focus on the five most commonly altered tumor suppressor genes identified in the PI's mouse model

Aim 2   Determine whether transcript levels of candidate tumor suppressor genes are reduced in SMNs

Specimens   Unstained, fixed sections of SMN samples Breast (n=48), Meningioma (n=23), CNS (n=18), sarcoma (n=10); paired normal samples

Funding   St. Baldrick's Foundation

Status   Samples currently being released and tested

Concept # 6   Genetic Epidemiology of Basal Cell Carcinoma in Childhood Cancer Survivors

PI   **Stella Davies, Cincinnati**

Genetic Epidemiology of Basal Cell Carcinoma in Childhood Cancer Survivors

Aims   Among cases who develop basal cell carcinoma , identify susceptibility polymorphisms in genes related to radiation sensitivity

Create a prediction model, using gene-environment interaction data for radiation sensitivity

Methods   Matched case-control study design

Candidate gene approach

Status   Funding: NIH (U01)

Genotyping largely done

Statistical analysis underway

Concept # 7   Susceptibility genes for radiation-induced breast cancer after Hodgkin lymphoma

PI   van Leeuwen/ Netherlands
Robison, Bhatia/ CCSS

Susceptibility genes for radiation-induced breast cancer after HL

Aim   Examine gene-environment interactions in patients with radiation-related breast cancer after HL

Design   Case-control study design

Cases: Caucasians with HL and secondary breast cancer

Controls: Caucasians with HL free of breast cancer until date of inclusion as controls

Matching criteria: age at dx of HL; calendar year of HL dx; length of f/u ≥ cases; exposure to supradiapgragmatic radiation

2nd comparison with available data on young BC without HL

Platform Illumina iSelect 200,000 targeted SNP chip

Status   Cases/ controls identified

Genotyping complete

Statistical analysis underway

Concept # 8   Genetic Susceptibility to Obesity after Childhood ALL

PI   Kala Kamdar, Baylor

Genetic Susceptibility to Obesity after Childhood ALL

Aims   Using GWAS, determine relation between treatment-related obesity and genetic polymorphisms (SNPS and CNVs) in 1200 ALL survivors from CCSS

Explore whether demographic and treatment variables modify relation between genotype and obesity in ALL survivors from CCSS

Replicate top SNPs and CNVs from discovery phase in an independent sample of at least 400 ALL survivors from TCH

Status   Funding procured (LLS)

GWAS analysis near-complete (as part of NCI GWAS initiative)

Concept # 9   Genetic Susceptibility to anthracycline-related CHF – Replication study

PI   **Smita Bhatia, City of Hope**

Genetic Susceptibility to anthracycline-related CHF Replication study

Aims   Replicate significant findings identified in the Discovery set (using COG-case-control study [ALTE03N1]) in an independent case-control set from CCSS

Status   Concept approved

Cases and controls identified for validation

Samples to be released post NCI GWAS effort

Concept # 10   Radiation-related thyroid cancer

PI   **Yuri Nikiforov, U of Pittsburgh**

Radiation-related thyroid cancer

Aim
Test whether alterations in DNA repair genes (*ATM, BLM, NBS1, DNA-PKcs, Ku70, XRCC4, RAD51*) lead to thyroid cancer in patients exposed to radiation

Design
Matched case-control study design

Matching criteria: Primary diagnosis, radiation field to involve thyroid, sex, age at exposure, race/ ethnicity, duration of follow-up to exceed latency between primary diagnosis and thyroid cancer

Status
Cases and controls identified

Samples to be released post NCI GWAS effort

Concept # 11   Genome-wide Association Study of Subsequent Malignant Neoplasms among Childhood Cancer Survivors (NCI/ CCSS)

NCI: L Morton, S Chanock, M Tucker, J Sampson, P Rajaraman

CCSS: S Bhatia, L Robison, M Stovall, G Armstrong, W Leisenring, Y Yasui, L Strong, J Neglia

Applications of Intent

AOI #1 Genetic Susceptibility to SMNs (Onel/ U of Chicago)

Specific Aim Test the association of top SNPs from the GWAS study with risk for second cancers after primary diagnoses other than HL

Eligibility criteria All patients with SMNs and controls 1:1 matched for primary cancer, latency, age of treatment for primary cancer, treatment modality, gender, and race/ethnicity

Status Concept is being finalized/ Request awaiting completion of primary analysis of NCI/CCSS GWAS

AOI #2 PRDM1 and somatic mutations in SMNs after HL (Onel)

Specific Aim Genotype chr 6q21 risk locus in all samples; assess (FISH / IHC) *PRDM1, MYC* mutation status in SMNs

Eligibility criteria Archival tumor samples of radiation-induced SMNs after HL

Status Concept is being finalized

AOI #3 Role of DNA repair genes in multiple SNs (Bhatia, City of Hope)

Specific Aim Examine the role of candidate genes involved in multiple SNs

Status AOI approved

Concept awaiting primary analysis of NCI/GWAS study

AOI #4 Epigenomic Profiling of Metabolic Outcomes Philip Lupo (TCCC)

Specific Aim 1: Determine if gene-specific DNA methylation status is associated with obesity in ALL survivors by conducting genome-wide DNA methylation profiling

Methodology *Characterize DNA methylation profiles using a genome-wide approach among 48 obese ALL survivors and 48 normal-weight ALL survivors*

Specific Aim 2: Identify obesity susceptibility genes in ALL survivors through an integrated genomic and epigenomic analysis. An integrative approach exploring genotype-epigenotype associations (INAS) will allow causal inferences about pathways contributing to obesity among ALL survivors.
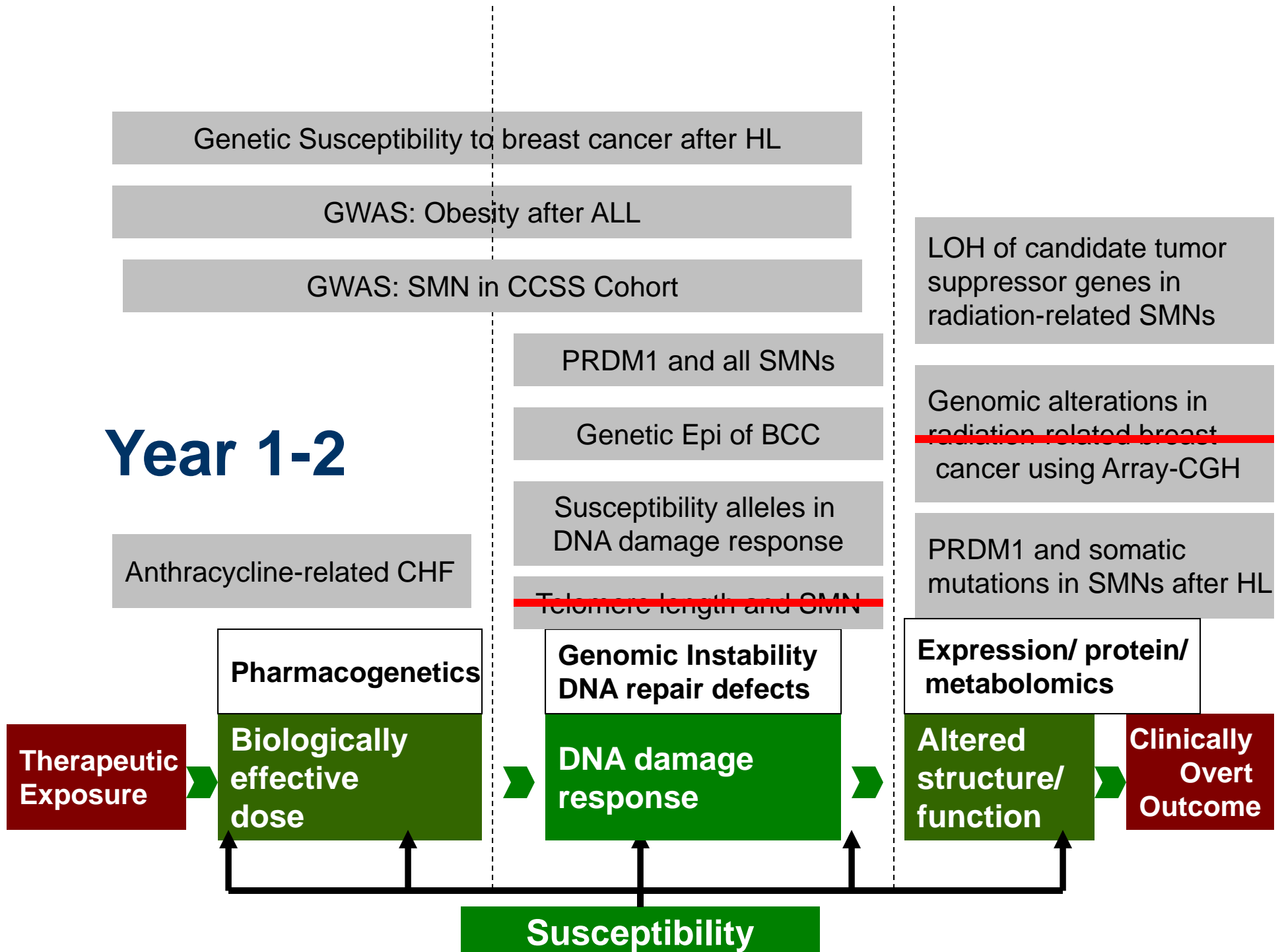
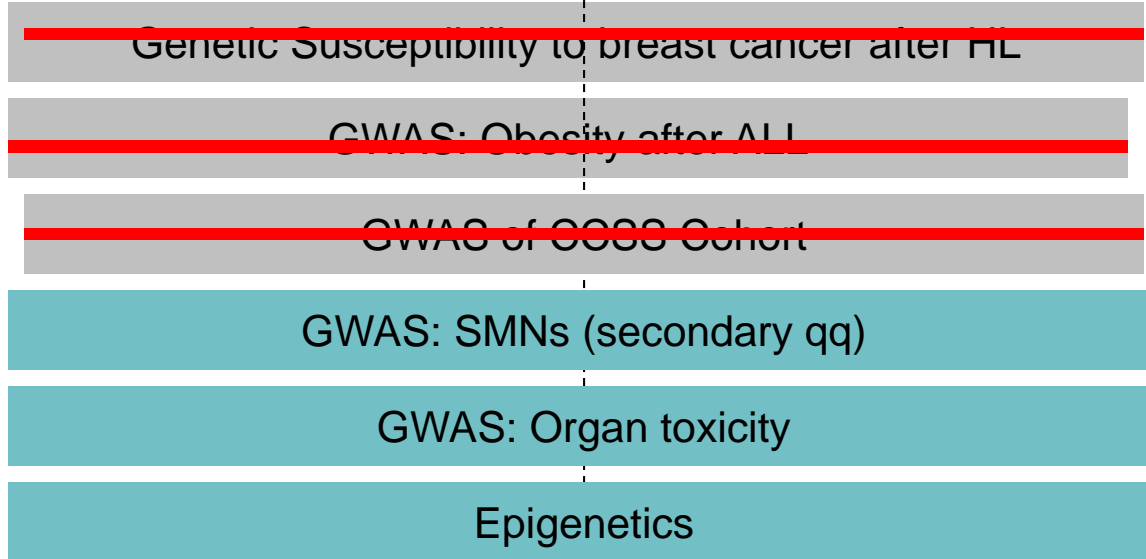*Methodology* *Perform methylation quantitative trait loci (mQTL) analyses to explore genotype-epigenotype associations.*
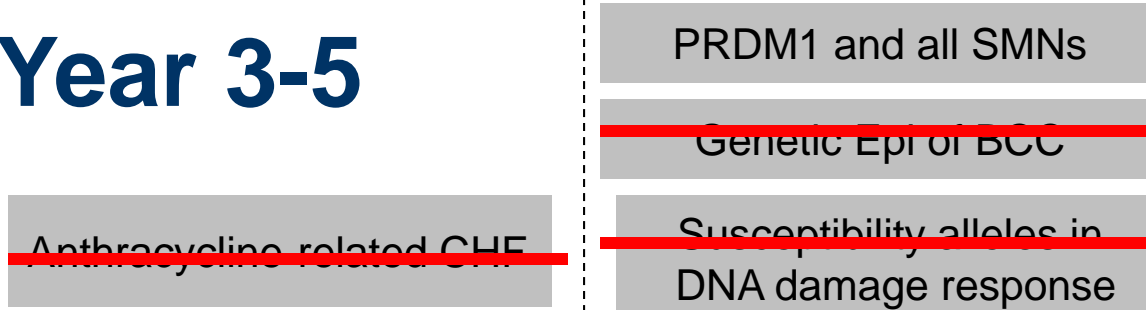
Status AOI approved

Concept awaiting decision regarding funding

Genetic Susceptibility to breast cancer after HL

GWAS: Obesity after ALL

GWAS of CCSS Cohort

GWAS: SMNs (secondary qq)

GWAS: Organ toxicity

Epigenetics

Year 3-5

PRDM1 and all SMNs

Genetic Epi of BCC

Susceptibility alleles in DNA damage response

Anthracycline-related CHF

Functional studies
In vitro
In vivo

Protein analysis
for candidate genes in
radiation-related SMNs

Gene expression analysis
in candidate genes in
radiation-related SMNs

LOH of candidate tumor
suppressor genes in
radiation-related SMNs

PRDM1 and somatic
mutations in SMNs after HL
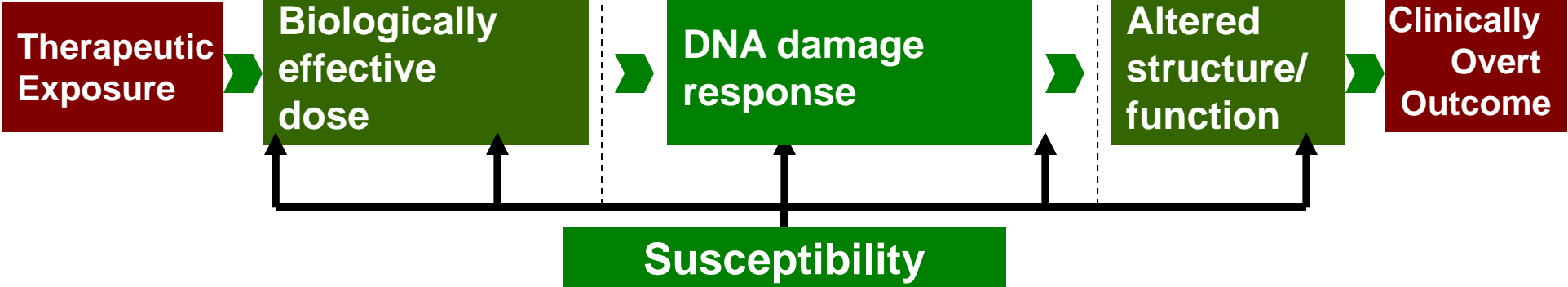
Therapeutic Exposure → **Biologically effective dose** → **DNA damage response** → **Altered structure/ function** → **Clinically Overt Outcome**

Susceptibility

GWAS

Organ toxicity

Hearing loss

- **Outcome of interest**
  - Hearing loss corrected with aid (n=187)
  - Deafness in both ears (n=21)

- **Therapeutic exposures**
  - Cranial radiation
  - Platinum compounds

- **Proposed/ published genetic associations**
  - *TPMT*
  - *COMT*
  - *GSTT1, GSTM1, GSTP1*

Congestive heart failure

- **Outcome of interest**
  - CHF requiring medications (n=140)
  - Cardiovascular death? (n=18)
  - Heart transplant (n=11)

- **Therapeutic exposures**
  - Anthracyclines
  - Radiation to the chest (involving the heart)

- **Proposed/ published genetic associations**
  - *NAD(P)H* oxidase NCF4
  - *CBR*
  - *HAS3*

Myocardial infarction

- **Outcome of interest**
  - Heart attack, angina or coronary heart disease (n=84)

- **Therapeutic exposures**
  - Radiation to the chest (involving the heart)

- **Proposed/ published genetic associations**
  - *MTHFR*
  - *Platelet glycoprotein IIb/IIIa*
  - *Nitric oxide synthase*
  - *Factor VII*
  - *Chemokine receptors*
  - *Angiotensin-converting enzyme (ACE)*
  - *Plasminogen activator inhibitor-1 (PAI-1)*

Gonadal dysfunction

- **Outcome of interest**
  - Ovarian failure (n=180)

- **Therapeutic exposures**
  - Radiation to the ovaries
  - Alkylating agents

- **Proposed/ published genetic associations**
  - Drug metabolizing enzymes
  - DNA repair

Stroke

- **Outcome of interest**
  - Stroke (n=127)

- **Therapeutic exposures**
  - Radiation

- **Proposed/ published genetic associations**
  - *MTHFR*
  - *ACE*
  - *Angiotensin (AGT) and angiotensin receptor type I (AGTR1)*
  - *Factor XIII-A*
  - *Lipoprotein lipase (LPL)*
  - *G-protein β-3 subunit (Gnbeta3 - 825 C/T) and α-Adducin (ADD1-G/W460)*

Cardiovascular Risk Factors – Diabetes

- **Outcome of interest**
  - Diabetes (n=57)

- **Therapeutic exposures**
  - Cranial radiation
  - Steroids
  - Abdominal radiation
  - TBI

- **Proposed/ published genetic associations**
  - **Diabetes:** *IGF2BP2, KCNJ11, NOTCH2, TCF7L2, TSPAN8 HHEX, HNF1B, IGF2BP2, IRS1, KCNJ11, KCNQ1, NOTCH2, PPARG, TCF7L2, THADA, TSPAN8, and WFS1*
  - **Obesity:** *LEPR, Gln223Arg*

Thyroid dysfunction

- **Outcome of interest**
  - Hypothyroidism (162)

- **Therapeutic exposures**
  - Cranial radiation

- **Proposed/ published genetic associations**
  - DNA repair genes – no published literature

Osteonecrosis

- **Outcome of interest**
  - Osteonecrosis with joint replacement (n=97)

- **Therapeutic exposures**
  - Site-specific radiation
  - steroids

- **Proposed/ published genetic associations**
  - Vitamin D receptor (*VDR*) gene
  - Plasminogen activator inhibitor-1 (*PAI-1*) gene
  - Thymidylate synthase (*TS*) gene
  - Collagen type I alpha gene

Cataracts

- **Outcome of interest**
  - Cataracts requiring surgery (n=39)

- **Therapeutic exposures**
  - Radiation
  - Prednisone

- **Proposed/ published genetic associations**
  - DNA repair

Pulmonary complications

- **Outcome of interest**
  - Lung fibrosis requiring oxygen(n=24)

- **Therapeutic exposures**
  - Chest radiation
  - Bleomycin
  - BCNU/CCNU
  - Busulfan
  - Cyclophosphamide

- **Proposed/ published genetic associations**
  - DNA repair genes

Request for use CCSS GWAS data

Application Process

- **PI proposing use of GWAS data must obtain permission from dbGap**
- **PI proposing to use GWAS data must submit the following to CCSS Data Access Committee (DAC)**
  - Data Access Request (DAR)
  - Data Use Certification

- **CCSS DAC reviews and prioritizes proposals based on**
  - Significance
  - Approach (methodological, technical and statistical)
  - Innovation
  - Compliance to CCSS priorities/ policies
  - Qualifications of the investigator team

- **Application material available on CCSS home page**
  - DAR forms
  - Instructions
  - Biosketch in NIH template
  - Data Use Certification/ CCSS policy document
  - Information regarding the review process

Data Use Certification

- **CCSS Policies and procedures for using the data**

  - Limiting use to project described in Data Access Request form

  - Not distributing data beyond those permitted to handle it

  - Not attempting to identify or contact study participants from whom phenotype data and DNA were collected

  - Awareness of principles regarding intellectual property

  - Adhering to policies on timeframe for publications stemming from data

  - Other provisions designed to protect confidentiality of study participants and foster scientific advance

Request for CCSS GWAS data

- All posted GWAS data have limited covariates linked to genotypes

- Individuals who receive GWAS data do not have access to CCSS ID for linkage to other covariate data

- No linkage with genotype data and other covariate data sets is allowed except by CCSS statistics committee

- Investigators may request data from a more comprehensive set of covariates
  - submit a proposal application

Attributable Risk

Attributable Risk

Background

- Clear association between therapeutic exposures and adverse outcomes
- Association modified by select demographic/ lifestyle factors
- Modification in therapeutic exposures over the decades

Objectives

- Develop a current perspective on the causes of premature morbidity and mortality attributable to cancer treatment vs. demographic vs. risk behaviors
  - Determine avoidable/ preventable causes of premature morbidity and mortality
  - Ascertain whether the attribution to treatment vs. modifiable risk factors has changed with the treatment era

Overarching Goal

- Comparative assessment of the contribution of potentially modifiable risk factors for key adverse events

Attributable Risk

Aims

- Describe premature cause-specific mortality **attributable to** key chemotherapeutic agents, health risk behaviors, BMI, differentiated by race, age and sex and **treatment era** for survivors of childhood cancer

- Examine chronic disease burden **attributable to** key chemotherapeutic agents, health risk behaviors, BMI, differentiated by race, age and sex and **treatment era** for survivors of childhood cancer, with a focus on two key health conditions:
  - Subsequent malignant neoplasms
  - Cardiovascular disease

Gaps in Knowledge

Gaps in Knowledge

- COG Long-term Follow-up Guidelines are risk-based, exposure-related clinical practice guidelines that provide recommendations for screening late effects

- The screening modality and intensity of screening are **consensus-based**

- NNCN scoring system has been adopted (with modifications)
  - Each score reflects the expert panel's assessment of the strength of data from the literature linking a specific late effect with a therapeutic exposure
  - Coupled with an assessment of the appropriateness of the screening recommendation based on the expert panel's collective clinical experience

- A total of 148 recommendations offered by the guidelines
  - Score 1A: 106 [72%]
  - Score 2A: 32 [22%]
  - Score 2B: 9 [6%])

- The score of 2A and 2B reflect a lack of rigorous evidence regarding the association between the therapeutic exposure and the outcome

Goal

- Identify gaps in knowledge regarding association between therapeutic exposure and outcome

  – Score 2A: 32 [22%]

  – Score 2B: 9 [6%])

- Data from original and expanded cohort will allow a wider range of exposures (in terms of doses), as well as access to the more contemporary exposures

Goal

Platinum compounds

- Male/ female gonadal dysfunction (2A)
- Therapy-related leukemia (2A)
- Peripheral sensory neuropathy (2A)
- Dyslipidemia (2B)

DTIC, Temozolomide

- Male/ female gonadal dysfunction (2A)
- Therapy-related leukemia (2A)

Alkylating agents

- Bladder malignancy (2A)

High dose cytarabine

- Neurocognitive deficit (2A)

6MP/ 6TG

- VOD (2A)

Radioiodine therapy

- Lacrimal duct atrophy
- Hypothyroidism

Methotrexate

- Renal toxicity (2A)
- Hepatic dysfunction (2A)
- Reduced BMD (2A)

Vinca alkaloids

- Peripheral sensory/ motor neuropathy (2A)
- Raynaud's phenomenon (2A)

Goal

- **Radiation**
- Metabolic syndrome (2A)
- Raynaud's phenomenon (2A)
- Subclavian artery disease (2A)
- Colorectal cancer (2A)
- Hemorrhagic cystitis (2A)
- Bladder malignancy (2A)
- Cholelithiasis (2B)
- Uterine vascular insufficiency (2B)

Hysterectomy
- Pelvic floor dysfunction (2A)
- Urinary incontinence (2A)

Pulmonary resection
- Pulmonary dysfunction (2A)
- Sexual dysfunction (2A)

Busulfan
- Cataracts (2B)

Radioiodine therapy
- Lacrimal duct atrophy
- Hypothyroidism

Bleomycin
- Pulmonary dysfunction (2B)

Dexamethasone/ prednisone
- Reduced BMD (2B)

HCT
- Reduced BMD (2B)

Oophorectomy (U/L)
- Premature menopause (2A)

Goal

Cystectomy

- Reservoir calculi (2A)

- Sexual dysfunction (2A)

- Spinal cord surgery (2A)

- Male/ female gonadal dysfunction (2A)

- Vitamin B12/ folate/ carotene deficiency (ileal entercystoplasty only) (2B)

Oophropexy

- Infertility (2A)

- Dyspareunia (2A)

- Symptomatic ovarian cysts (2A)

- Bowel obstruction (2A)

- Pelvic adhesions (2A)

Thank you

Questions? Comments?