CCSS
An NCI-funded Resource

Childhood Cancer Survivor Study (U24 CA55727)

Report of the Genetics Working Group

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Genetics Working Group

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June, 2010

Topics

1. Strategies for procurement of samples
2. Management of biospecimens
3. Process for release of Biospecimens
4. Status of Concepts/ AOIs

Genetics Workshop

Strategies for procurement of samples

Overall consensus….

….We want “more”

Oragene

- Entire cohort
  - **May consider procuring two specimens/subject**

Strategies for procurement of samples

Blood

- Prioritization:
  - Patients with high mortality
    - » go back to these soon for blood and LCL
  - Patients with adverse outcomes
    - » CTCAe grade >2
  - Patients with SPCs
    - » More than one second primary
    - » Second primary without XRT
    - » Family history of cancer in first degree relative before age 40
    - » Second primary after a short latency from XRT (<10 years)

Strategies for procurement of samples

- **SMN tissue**
  - High priority for CGH arrays
  - QA on tissue specimens
    - » H & E stains to ensure adequate malignant tissue
  - Paired normal tissue desirable
  - Flag case/ block and at community hospital
    - » they will send materials when ready to trash

Oragene samples – Current status

Methodology

- Send letter, consent form, kit to active participants
- Trace non-current addresses/ resend kit if address found
- Call until contact or until reminder message left
- Resend kit upon participant request
  - Call intensity increased in Dec, 2009 (up to 10 calls/ participants)
  - Resend kit upon participant request
  - Mass resend to all non-responders occurred in January 2010

Survivors (n = 8,268)	
Return rate:	3066 (37%)
Refusal:	308 (3.7%)
Pending:	4659 (56%)
In tracing:	109 (1.3%)
Deceased	22 (0.3%)

Siblings (n = 2,710)	
Return rate:	701 (26%)
Refusal:	76 (2.8%)
Pending:	1923 (71%)
In tracing:	109 (1.3%)
Deceased	10 (0.4%)

Procurement of Blood/ Tissue – Current status

Eligibility criteria: Confirmed SMNs (NMSCCs excluded)

Letters sent prior to FU2007	n=621
Passive refusals	n=183 (29%)
Active refusal	n=47 (7.6%)
Pending	n=23 (3.8%)
Deceased	n=35 (5.6%)
Consents returned	n=333 (53.6%)
Agreed to blood only	n=11
Agreed to blood and tissue	n=300 (48.3%)
Agreed to tissue only	n=22
Blood specimens received	**n=280 (45.1%)**
Tissue specimens received	**n=195 (31.4%)**

Letters for blood draws sent to 264 patients with confirmed SMNs

Genetics Workshop

Management of biospecimens

- Quality of biospecimens
  - 260/280 ratios for DNA extracted from buccal cells/ Oragene
- Quantity of biospecimens
- Collect specimens in “straws” to maximize space and also minimize “freeze/thaw” cycles
- Biopsecimen tracking database to maintain accurate record of quantity, nature of biospecimens

Total number of Samples

All survivors

- Buccal cells 6954
- Oragene 3535
- Blood 991

SMNs

- Buccal cells 1237
- Oragene 742
- Blood 574

Advertisement, Promotion of Biospecimens

- **Query tool on the CCSS website**
  - Number of subjects with specimens
  - Demographic characteristics
  - Clinical characteristics
  - Type of specimens
    - » blood/ buccal cells/ tissue
  - Quantity of specimens available

Process for release of Biospecimens

- **Concept to include following**
  - Hypothesis, aims
  - Background and rationale
  - Study design
  - Sample size and power
  - Funding source

Biospecimen Advisory Committee

- Advise appropriate release of biospecimens
  - Scientific merit
  - Technical merit
  - Statistical merit
- Members of Advisory Committee
  - Molecular/ genetic epidemiologist
    - Susan Neuhausen, PhD
    - Stephen Gruber, PhD
  - Statistical Geneticist
    - Yutaka Yasui, PhD
  - Genetics Working Group Co-Chairs
  - Ad hoc members
    - Relevant working group chairs

Process for release of Biospecimens

- Prioritization for release of specimens
  - Availability of specimens
  - Scientific and technical merit
  - Extramural funding

Concepts/ AOIs

Status

June, 2010

Concept	Date AOI approved	Date Concept approved	Status
aCGH and radiation-related breast cancer	6/08	Approved	aCGH analysis complete
Genetic Epi of BCC			Cases/ controls identified (3/10) Genotyping underway (5/10)
DNA damage response pathway and radiation-related breast cancer			R01 funded project
GWAS of SMNs after HL			R21 funded MS submitted
Telomere length and SMN	7/09	1st submission: 4/10 2nd submission: 6/10	K12 funding

Concept	Date AOI approved	Date Concept approved	Status
SNPs in the EWS Breakpoint Region	12/08	Approved	Genotyping complete; Statistical analyses underway K12 funding
Genetic Susceptibility to Obesity after ALL	8/08	Approved conditional on funding	Submitted for funding to LLS 4/10

AOI	Date AOI approved	Concept?	Status
Genetic predictors of neuropsychological functioning (lymphoma/ leukemia)	3/09	In process	
Late effects in Childhood Cancer Survivors and Vitamin D Polymorphisms	3/09	6/10	Under review by Biospecimen Advisory Committee
Long-term morbidity in childhood cancer survivors with DS	12/08	4/1/10	Under review
Polymorphisms of cardiomyocyte genes in anthracycline-related CHF or subclinical left ventricular dysfunction	11/09		List of candidate SNPs being finalized

AOI	Date AOI approved	Concept?	Status
Genome-wide association study and second cancers	11/09	In process	
Breast cancer after HL: Genetic Susceptibility Dutch/ UK/ CCSS	Under discussion		

Concept # 1 Characterization of genomic alterations in radiation-related breast cancer using Array-CGH (Comparative Genomic Hybridization) Yang/NCI

- **Aims**
  - To estimate the prevalence and patterns of DNA copy number changes in radiation-exposed breast tumor tissues obtained from participants in the Life-span study (n=50) and CCSS (n=35) using array-CGH
  - To identify distinct genomic aberrations related to radiation exposure and doses by comparing CGH profiles among breast tumors exposed to high-dose, low-dose, and no radiation (LSS=25; CCSS=10)
  - To examine the relationship between copy number changes and age at exposure and type of radiation exposures (acute vs. protracted).

Concept # 1 Characterization of genomic alterations in radiation-related breast cancer using Array-CGH (Comparative Genomic Hybridization) Yang/NCI

- **Status**
  - AOI approved (6/08)
  - Concept approved
  - 50 breast cancer cases
    - 10 unstained tissue sections per case
  - P Meltzer's lab completed DNA extraction and aCGH analysis
    - Insufficient DNA (n=14); poor quality DNA (n=7)
  - Requested additional sections for 21 cases
    - Lab completed the array CGH analysis (3/10)

Concept # 1 Characterization of genomic alterations in radiation-related breast cancer using Array-CGH (Comparative Genomic Hybridization) Yang/NCI

- **Status**
  - Analysis of 32 CCSS samples with aCGH data; reasonable quality
  - Typical breast cancer CGH profile is observed
    - More amplifications and complex changes
    - Changes do not correlate with radiation exposure
  - Number of non-exposed cases limited
    - ~5
  - Searching for published aCGH data for reference samples

Concept #2 Evaluation of SNPs in the EWS Breakpoint Region in People with and without Ewing sarcoma (DuBois/UCSF)

- **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 the entire *EWSR1* gene
- **Secondary Aims**
  - Determine if frequency of SNPs in the *EWSR1* gene differs between patients with Ewing sarcoma who survived 5 years from initial diagnosis and those who did not
  - Determine if frequency of SNPs in the *EWSR1* gene differs between patients with Ewing sarcoma and siblings of patients with Ewing sarcoma
  - Determine frequency of any SNPs associated with Ewing sarcoma in a control population of African ancestry

Concept #2 Evaluation of SNPs in the EWS Breakpoint Region in People with and without Ewing sarcoma (DuBois/UCSF)

- **Status**
  - Concept approved/ ancillary study approved
  - Samples received and genotyped
  - Statistical analysis underway (5/31/10)
  - Sequence intron 7 to identify possible novel SNPs that might be associated with Ewing sarcoma

Concept #3 Genetic Susceptibility to Obesity after Childhood Leukemia (Kamdar/ Baylor)

- **Aims**
  1. Using GWAS, determine relation between treatment-related overweight/obesity and genetic polymorphisms (SNPS and CNVs) in 1200 ALL survivors from CCSS
  2. Explore whether demographic and treatment variables modify relation between genotype and overweight/obesity in ALL survivors from CCSS
  3. Replicate top SNPs and CNVs from discovery phase of GWAS in an independent sample of at least 400 ALL survivors from Texas Children's Hospital, using targeted genotyping of promising SNPs or CNVs with a custom genotyping panel
- **Status**
  - Revised concept submitted to LLS for funding
    - Outcome expected in June, 2010

Concept #4 Genetic Epidemiology of Basal Cell Carcinoma in Childhood Cancer Survivors (Davies/Cincinnati)

- Aims
  - Using a matched case-control design, identify susceptibility polymorphism frequencies in genes related to radiation sensitivity among cases who develop basal cell carcinoma
  - Create a prediction model, using gene-environmental interaction data for radiation sensitivity in children diagnosed with primary ca
- Methods
  - Candidate gene approach/ matched case-control study design
- Status
  - Funding: NIH (U01)
  - Cases and controls identified (3/10)
  - Genotyping underway (5/10)
    - Spent some time cleaning up old DNA; new DNA much better in quality

Concept #5 Environmental Exposure: Susceptibility alleles in DNA damage response pathway (Stambrook/ U of Cincinnati)

- Study aims
  - Define genetic changes in the signaling pathway that contribute to susceptibility to breast cancer after radiation
- Rationale
  - DNA damage results in cell cycle checkpoint arrest and repair of the damage or elimination of the damaged cells
    - DNA damage response proteins activated
      - ATM CHEK2, Cdc25A, Plk3, BRCA1 and BRCA2
- Methods
  - Mouse models (knockout)
  - Human cohorts – radiation-related breast cancer
    - CCSS
    - Fernald Uranium processing plant with breast cancer
- Status
  - Funding: NIH (1 R01 ES016625-01A1)

Concept #6 GWAS of second cancers after HL

Onel/ U of Chicago

- Funding: NIH (R21)
  - GWAS in patients treated for HL
    - Discovery set
      - 103 cases with SMNs and 121 controls
    - Replication set
      - 120 cases and 112 controls without SMNs
  - Results
    - Genes identified in Discovery set
    - Validated in Replication set
  - MS ready for submission

Concept #7 Telomere length and Second Malignancy in Pediatric Cancer Survivors (Gramatges/ Baylor)

Hypothesis Shortened germline telomere length may play a role in development of second cancers in pediatric cancer survivors

- increased likelihood for mutation gains or losses in an already strained checkpoint system

Aims Using a matched case-control study design, investigate relation between mean telomere length in buccal DNA samples and second malignancy in childhood cancer survivors

Status

AOI approved (7/09)

Concept submitted – concerns raised (4/10)

Concept revised and resubmitted (6/8/10)

AOI # 1 Genetic predictors of impaired and enhanced neurocognitive and psychological functioning among survivors of childhood leukemia and lymphoma (Kadan-Lottick/ Yale)

- Status
  - AOI approve (3/09)
  - Concept to be submitted soon

AOI # 2 Late effects in Childhood Cancer Survivors and Vitamin D Polymorphisms (Ocku/ TCCC)

- Status
  - AOI approved (3/09)
  - Concept submitted (6/10)
  - Under review by Biospecimen Advisory Committee

AOI #3 Long-term morbidity in childhood cancer survivors with DS (Raber/ UCSF)

- Aims
  - Describe incidence and risk factors for morbidities in leukemia survivors wit DS
  - Compare incidence and nature of morbidity in leukemia survivors with and without DS
- Status
  - AOI approved (12/08)
  - First version of analysis concept submitted (4/1/10)

AOI #4 Polymorphisms of cardiomyocyte genes in CCSS participants with CHF or subclinical left ventricular dysfunction after treatment with anthracyclines

Green/ SJCRH

- Aims
  - Determine if frequency of polymorphisms in one or more genes known to be associated with familial dilated cardiomyopathy is increased in patients with impaired left ventricular function related to anthracycline treatment
- Status
  - AOI approved (11/09)
  - Concept under preparation
    - SNPs to be identified
    - Sample size/ analytic plan to be finalized

AOI #5 Genome-wide association study and second cancers

Preetha Rajarama/ NCI

- Status
  - AOI approved (11/09)
  - Concept under preparation

AOI #6 Genetic susceptibility to breast cancer after HL

van Leeuwen/ Netherlands; Zwerdlow/ UK; Robison/ CCSS

- Status
  - AOI under discussion