CCSS
Childhood Cancer Survivor Study

Genetics Working Group

Smita Bhatia

St. Jude Children's Research Hospital

An NCI-funded Resource

Working Group Membership

CCSS

- Greg Armstrong
- Smita Bhatia
- Stephen Chanock
- Diana Merino
- Lindsay Morton
- Les Robison
- Joshua Sampson
- Peggy Tucker
- Lucie Turcotte
- Yutaka Yasui

- Conference calls
  - Wednesdays at 12:30 pm Central Time

Genetics

CCSS

- Current status of CCSS-related activities
- Strengths, limitations, opportunities

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Genetics WG – Current status of CCSS-related activities

CCSS

1) SNP array data
    1) 5,739 childhood cancer survivors diagnosed 1970-1986 (5,324 of European ancestry)
    2) Illumina HumanOmni5Exome microarray
    3) over 4.1 million loci passed quality control thresholds

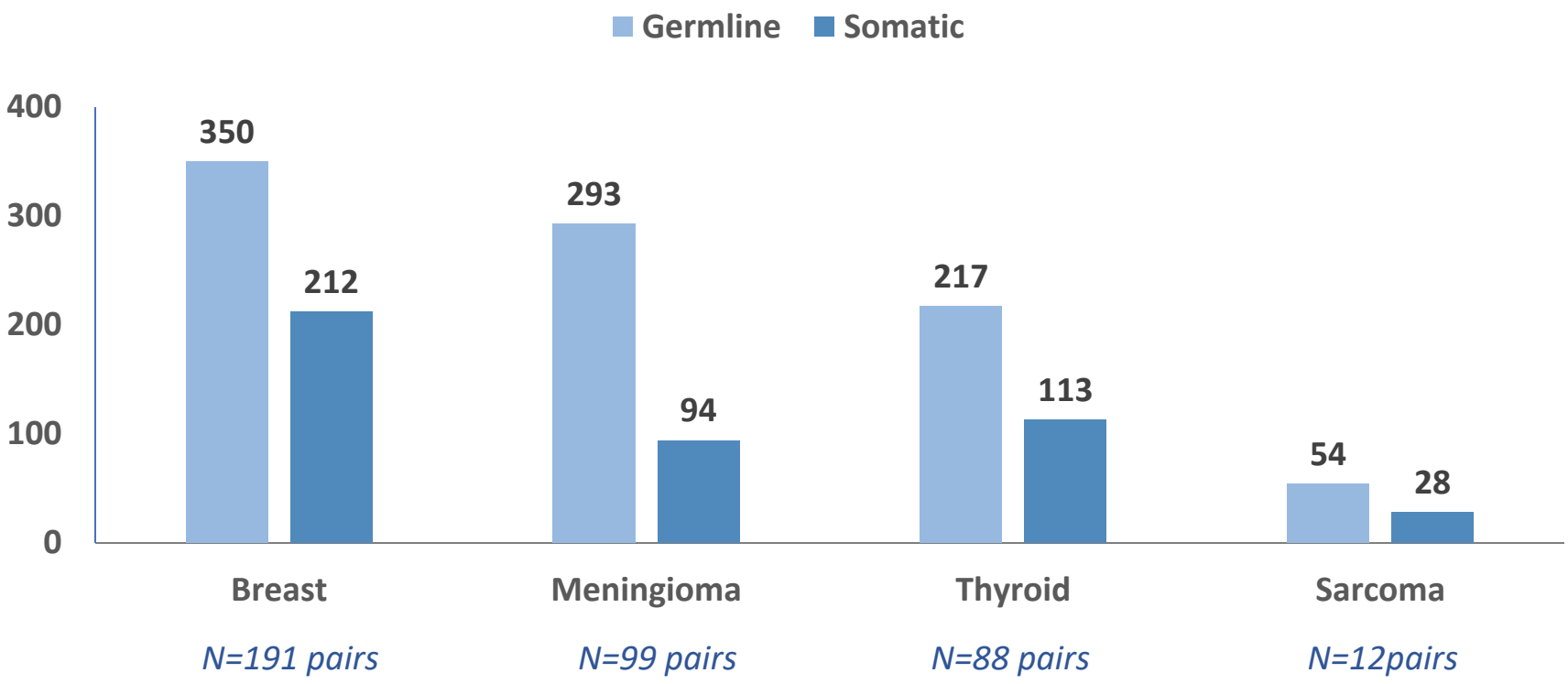
2) WES data for ~8400 childhood cancer survivors diagnosed 1970-1999

3) WGS data for ~3000 childhood cancer survivors diagnosed 1987-1999

4) Accompanying phenotype data

Genetics WG – Current status of CCSS-related activities

CCSS



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Genetics WG – Current status of CCSS-related activities

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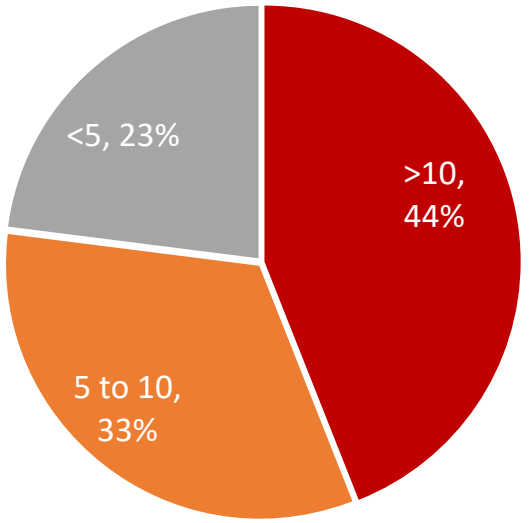
Published/In Press Manuscripts (2018/2019) n=9

Nat Commun. 2018;9:3184
BMC Genomics. 2018;19:182
Cancer. 2018;124:617-25
J Natl Cancer Inst. 2018;110:895-904
Genes Chromosomes Cancer. 2019;58:52-9
Cancer Epidemiol Biomarkers Prev. 2019;28:417-9
J Inv Dermatol, 2019, doi: 10.1016/j.jid.2019.02.029. [Epub ahead of print]
Blood. 2019 Mar 7;133(10):1130-1139
J Clin Oncol, 2019, (in press)



Analyses in Progress n=13

Ancillary studies n=5

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Genetics WG – Current status of CCSS-related activities
Concepts

CCSS

#	Outcome of interest	PI	Replication	Status
1	SMNs	Morton	SJLIFE	Analyses underway
2	Stroke, MI	Morrison/ Bowers	UTSW, SJLIFE	Replication underway
3	Diabetes	Lupo	SJLIFE, CCSS expansion	Replication being planned
4	Tinnitus	Dolan	SJLIFE	Discovery analysis underway
5	Cardiomyopathy	Bhatia	ALTE03N1, SJLIFE	Analysis underway
6	Candidate anthracycline-responsive genes as predictors of cardiotoxicity	Reyes/ Hildebrandt	MDACC	Analysis underway

Genetics WG – Current status of CCSS-related activities
Concepts / AOI

CCSS

#	Outcome	PI	Replication	Status
8	Pregnancy	*Rotz/ Kuo*	SJLIFE	Analysis underway
9	Fracture	*Im*	SJLIFE	Analysis underway
10	Neurocognitive impairment	Scheurer	SJLIFE	Concept approved
11	Post-traumatic stress	*Recklitis*	SJLIFE	AOI approved
12	Hearing loss	*Dolan*	SJLIFE	AOI approved

Working Group Progress – Concept #1

CCSS

GWAS of Stroke and Myocardial Infarction: Morrison/Bowers Univ. Texas Health Science Center

Aims

- Identify *common* genetic variants associated with stroke and MI
- Identify *low- and intermediate-frequency* genetic variants associated with stroke and MI

Discovery Cohort

MI: n=3956 (73 with MI)

Stroke: n=3968 (186 with stroke)

Replication: SJLIFE and CCSS Expansion cohort

Status: Analysis is currently underway

Preliminary analysis: No variants associated with MI or stroke reached p-value of $<5x10^{-8}$
Seeking to replicate 8 common variants with a P-value $<1x10^{-7}$ in the *CCDC141* gene on chromosome 2 associated with MI, 4 additional index SNPs associated with MI; 11 common variants associated with stroke.

All loci are additionally supported by corroborating evidence from GWAS for clinical phenotypes that were conducted in the UK BioBank or large genomic consortia such as Cardiogram+C4D and MegaStroke

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Working Group Progress – Concept #2

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Genetic Architecture of Diabetes Mellitus in Long-term Survivors of Childhood Cancer: *Lupo, TCCC*

Specific Aims

1. Evaluate the role of previously-published *de novo* type 2 DM genetic variants in the risk of DM
2. Identify novel genetic variants associated with DM (GWAS)
3. Develop and validate an integrated clinical and genetic risk prediction model for DM

Discovery Cohort – n=4,804 (360 with diabetes)

Replications

- SJLIFE, CCSS Expansion

Status

Aim 1: abstract submitted to NASLCCC

Aim 2: analysis in progress; GWAS discovery completed; pending replication

Aim 3: concept under review

Working Group Progress – Concept #3

CCSS

Tinnitus – GWAS study; *Dolan/ U of Chicago*

- **Aim 1:** To perform: 1) a GWAS in pediatric cancer survivors; and 2) a genome-wide meta-analysis of cisplatin-induced tinnitus in the testicular cancer survivor cohort and the pediatric cancer survivors.
- **Aim 2:** To perform PrediXcan, providing directionality of effect and potential molecular mechanisms in pediatric cancer survivors.
- **Aim 3:** To identify associations between identified genes and PheWAS (ICD-9-CM) codes for tinnitus in BioVU, Vanderbilt's DNA biobank linked to electronic medical records.

Status: SNPs near *DCAF6* and intronic to *MBD5* were identified to be associated with radiation-induced tinnitus

Working Group Progress – Concept #4

CCSS

Pathogenesis of anthracycline-related cardiomyopathy in childhood cancer survivors: Bhatia/ UAB

Specific Aims

- **Aim 1: Identify genetic variants associated with anthracycline-related cardiomyopathy**
  - ***Aim 1.1:*** *Identify novel genetic variants*
  - ***Aim 1.2:*** *Characterize the role of previously-published genetic variants associated with anthracycline-associated cardiomyopathy*
- **Aim 2:** Define **functional relevance** of genetic variants using human-induced pluripotent stem cell-derived cardiomyocytes and human myocardial tissue.
- Sample size: 220 cases/5096 controls
- Replication: COG-ALTE03N1

Status: Phenotype and genotype data received. Aim 1.2 completed; abstract presented at ASCO and NASLCCC

Working Group Progress – Concept #5

CCSS

Analysis of Genetic Variants from Candidate Anthracycline-responsive Genes in iPSC-Cardiomyocytes as Predictors of Cardiotoxicity: *Reyes/ Hildebrandt MD Anderson Cancer Center*

Specific Aims

- In 148 genes identified from an analysis of anthracycline response in iPSC-cardiomyocyte RNAseq data, identify variants associated with risk of cardiotoxicity in CCSS
- Identify genetic variants in genes modulated in iPSC-cardiomyocytes during doxorubicin exposure that can predict risk of cardiotoxicity stratified by cumulative doses

CCSS cohort: Anthracycline-exposed survivors: N=1,912; Chest radiation-exposed survivors: N=3,261

Replication: Childhood cancer survivors at MDACC (N=700).

Status: Concept approved; phenotype and genotype data with PI; analysis underway

Working Group Progress – Concept #6

CCSS

Intestinal obstruction: *Madenci/ Boston Children's*

- **Specific Aims**
  - To identify genetic variants associated with the development of late intestinal obstruction requiring surgery (IOS) in a candidate gene analysis
  - To identify genetic variants associated with the development of late intestinal obstruction requiring surgery (IOS) in a genome-wide association study (GWAS).

CCSS cohort: 4027 survivors; intestinal obstruction (n=88)

Results: no variants reached p-value of $5x10^{-8}$; several close

Replication: replication GWAS in SJLIFE

Working Group Progress – Concept #7

ccss

Genetic susceptibility to neurocognitive impairment: *Scheurer/ Baylor*

- **Specific Aims**
  - Characterize novel genetic variants associated with neurocognitive functioning domains in childhood cancer survivors.
  - Replicate and functionally annotate novel genetic variants associated with neurocognitive functioning and its domains in childhood cancer survivors.

CCSS cohort: 3,592 survivors

Replication: SJLIFE

Status: Concept approved (3/6/2019); analysis underway

Ancillary Studies (1)

CCSS

Principal Investigator: Kala Kamdar (Baylor)

Title: Genetic Susceptibility to Obesity after Treatment for Childhood Leukemia

Funding Source: Leukemia & Lymphoma Society Translational Research Award

Study Aims: Using genome-wide and candidate pathway analyses, to identify genetic variants involved in the development of obesity.

Current Status: Finalized the analysis in CCSS and submitted 23 SNPs that were $P<10^{-7}$ for replication in SJLIFE. One SNP was $P<0.05$ in SJLIFE. CCSS Expansion replication is underway. Currently finalizing the replication analyses and drafting manuscript

Ancillary Studies (2)

CCSS

Principal Investigator: Jennifer Yeh (Harvard Medical School)

Title: Genetic Testing to Guide Pediatric Cancer Care and Follow-up: Using Anthracycline-Associated Cardiomyopathy as a Model for the Future

Dates of Funding: 9/18 - 8/23

Funding Source: National Institutes of Health (Provocative Questions R01)

Study Aims: Use simulation modeling to: 1) determine the clinical impact of utilizing genetic variant testing for cardiotoxicity in guiding cancer care, 2) assess how consideration of genetic markers can improve follow-up cardiac screening recommendations for at-risk survivors.

Current status: Notice of Award – 9/2018 – work is currently underway…

Ancillary Studies (3)

CCSS

Principal Investigator: M Monica Gramatges (Baylor College of Medicine)

Title: Shortened telomere length and defects in telomere maintenance associated with thyroid second malignant neoplasm in childhood cancer survivors

Funding Source: National Institutes of Health (R01)

Aim 1: Determine whether SNPs associated with telomere maintenance are enriched in survivors with thyroid SMN (GWAS) – CEBP

Aim 2: To examine telomere content using Flow FISH and understand impact of defects in telomere maintenance genes upon telomerase function

Ancillary Studies (4)

CCSS

Principal Investigator: Jean Nakamura (University of California, San Francisco)

Title: Neurofibromin and response to genotoxins

Dates of Funding: 7/12 - 6/15

Funding Source: St. Baldrick's Foundation

Aim 1: Determine whether loss of heterozygosity in tumor suppressor genes identified in the *Nf1* mutant mouse model occurs in SMNs of childhood cancer survivors

Clin Cancer Res. 2017 Apr 1;23(7):1852-1861

Aim 2: Determine whether transcript levels of candidate tumor suppressor genes are reduced in second malignant neoplasms.

Tissue quality inadequate to complete this aim

Ancillary Studies (5)

CCSS

Principal Investigator: Smita Bhatia (UAB)

Title: Developmental HyperActive Ras Tumor SPORE

Dates of Funding: 7/15 - 6/20

Funding Source: National Institutes of Health (SPORE)

Study Aims: to describe risk of SMNs and demographic/therapeutic factors associated with SMNs in individuals with NF1 and primary cancer

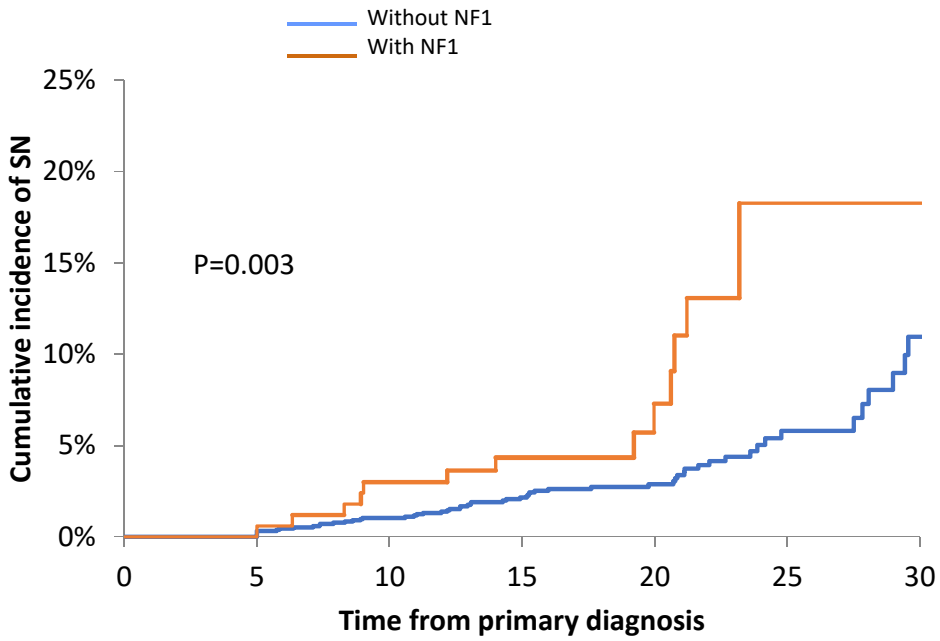
Current status: JCO in press, 2019

SPORE renewal underway.

Highlights of Recently Completed Research

CCSS

Hypothesis 1: Risk of SNs will be higher in childhood cancer survivors with NF1 when compared those without NF1, independent of therapeutic exposures.



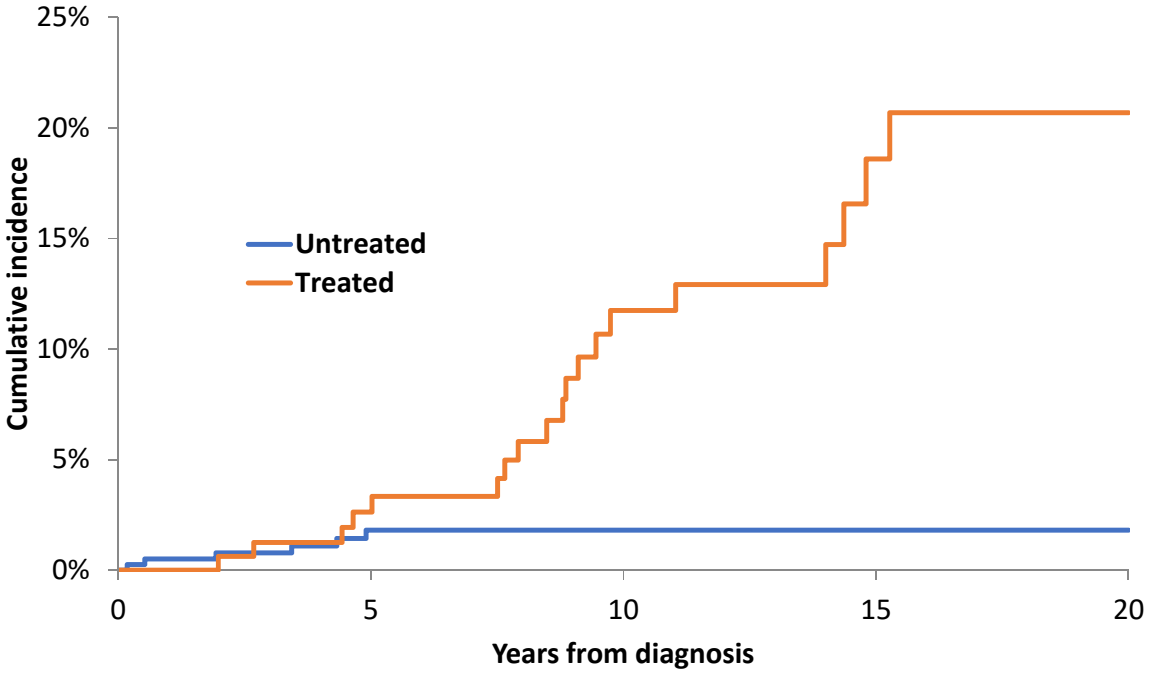
Variables	HR	95% CI	P-value
No NF1	1.0		
NF1+ (SN)	2.4	1.3-4.3	0.005
NF+ (SMN)	3.5	1.7-6.5	<0.001

Proportional Subdistribution Hazards Regression for Survival Analyses with Competing risks, adjusted for age at dx, race, primary cancer, anthracyclines, alkylating agents, radiation

Highlights of Recently Completed Research

CCSS

Hypothesis 2: Among NF1-affected children with a primary tumor, the risk of SNs in the treated cohort will be higher when compared with the untreated cohort.



NF1 patients without treatment (n=441)	REF	95%CI	P-value
NF1 patients with treatment (n=176)	6.1	(2.6-14.1)	<0.001

JCO, in press, 2019

Highlights of Recently Completed Research

CCSS

Hypothesis 3: Among the treated NF1-affected children with primary tumor, the risk of SNs will be higher for those exposed to radiation and/or alkylating agents when compared with those treated with other therapies.

Variables	Univariable			Multivariable*		
	HR	95% CI	P-value	HR*	95% CI	P-value
Therapeutic exposures						
Anthracyclines (n=11)	1.35	(0.2-9.08)	0.8			
Platinum compounds (n=48)	0.57	(0.3-1.3)	0.2			
Alkylating agents (n=97)	1.51	(0.7-3.5)	0.3			
Radiation (n=24)	2.94	(1.3-6.9)	0.01	2.78	(1.28-6.03)	0.01

JCO, in press, 2019

Genetics

CCSS

- Current status of CCSS-related activities
- **Strengths, limitations, opportunities**

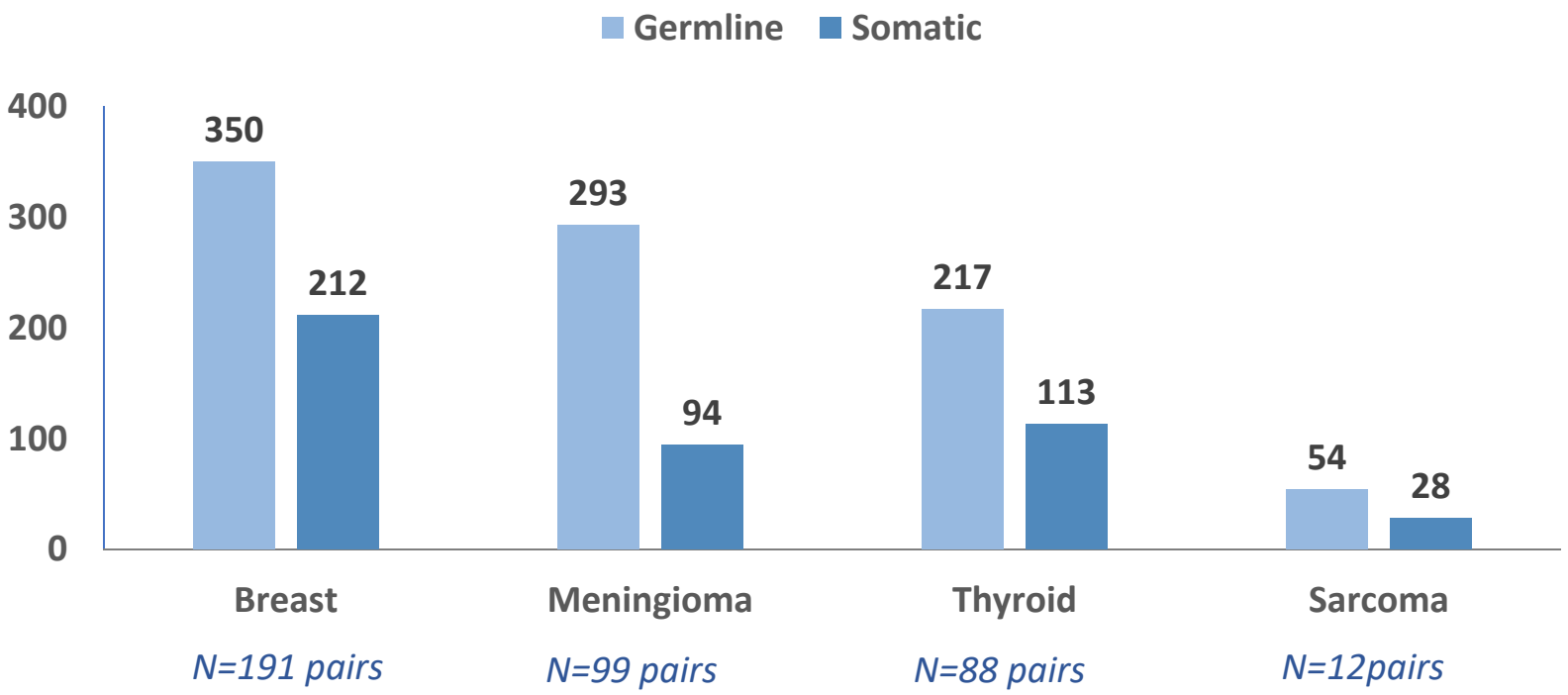
Genetics WG – Strengths

CCSS

1) Genotype data for **5739** childhood cancer survivors diagnosed 1970-1986
    1) Illumina HumanOmni5Exome microarray
        1) > 4.1 million loci passing QC thresholds.
    2) 5324 of European ancestry
    3) 415 of non-European ancestry
2) WES data for **~8400** childhood cancer survivors diagnosed 1970-1999
3) WGS data for **~3000** childhood cancer survivors diagnosed 1987-1999
4) Accompanying phenotype data

Genetics WG – Current status of CCSS-related activities

CCSS



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Genetics – limitations

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i) Self-reported outcomes (without source validation)
   i) Exception: SMNs
   ii) Certain outcomes more susceptible to misclassification

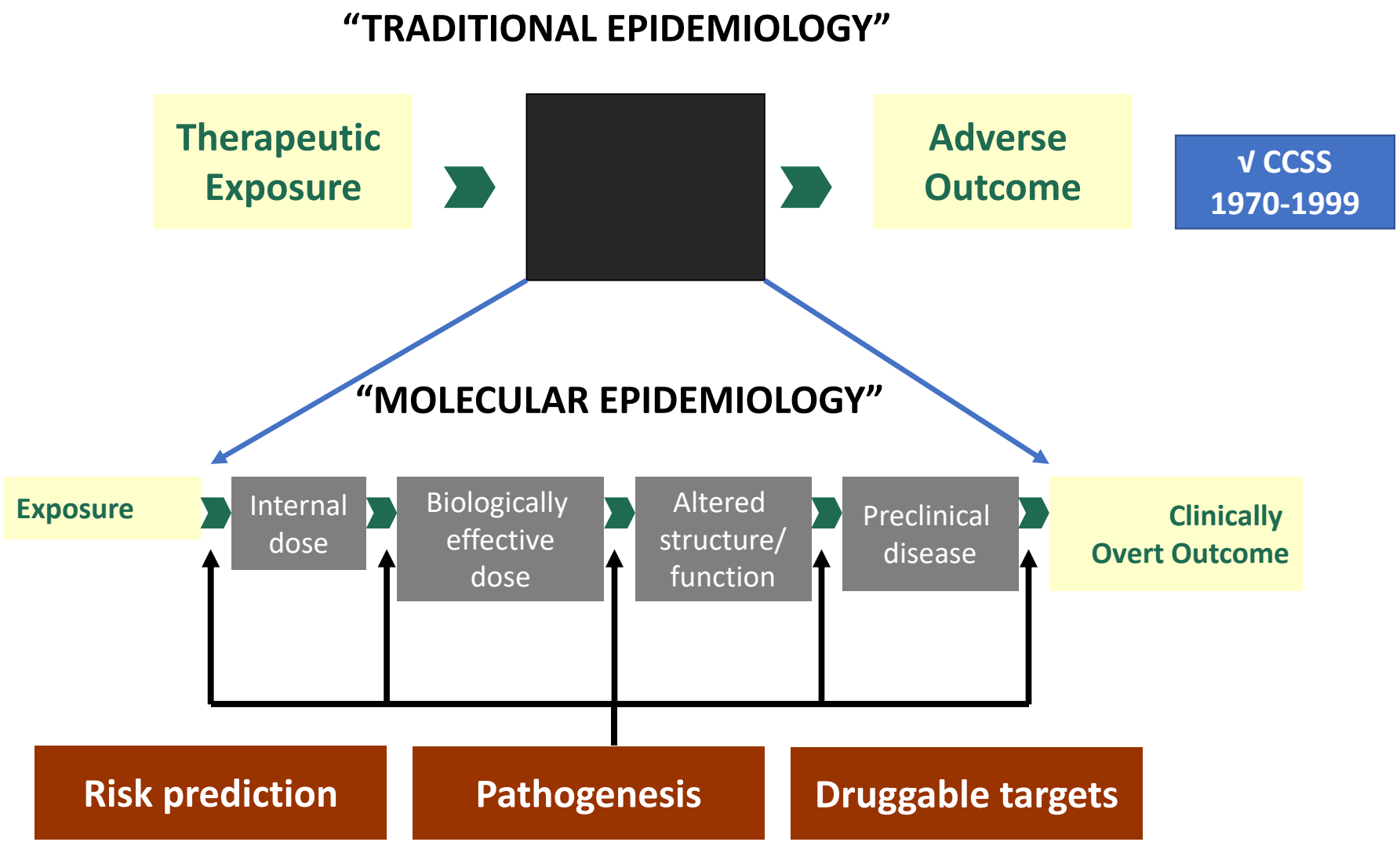
ii) Limited number of patients with outcomes (n=73 [MI] to n=360 [Diabetes])
   i) Sample size may not be adequate to determine rarer outcomes/ association with rare variants

iii) Small absolute numbers of racial/ethnic minorities with sequencing/ genotype data

iv) Somatic tissue – quality of extracted nucleic acids

v) Limited options for replication

vi) Limited expertise in analyzing/ interpreting genetic data


"TRADITIONAL EPIDEMIOLOGY"
Therapeutic Exposure
Adverse Outcome
√ CCSS 1970-1999
"MOLECULAR EPIDEMIOLOGY"
Exposure
Internal dose
Biologically effective dose
Altered structure/ function
Preclinical disease
Clinically Overt Outcome
Risk prediction
Pathogenesis
Druggable targets

Genetics WG – Opportunities

CCSS

1. Utilization of this resource to determine molecular pathogenesis of treatment-related complications
    1. GWAS, WES, WGS data
    2. Paired germline DNA/ SMN tissue
2. Develop integrated risk-prediction models
3. Druggable targets
4. Extend beyond genomics to other “omics”
    1. Transcriptomics
    2. Proteomics
    3. Metabolomics
    4. Microbiome

Genetics – Threats

CCSS

1. 5y survivors – survival bias
2. Representativeness of the sample cohort to the overall CCSS cohort
3. Need for large replication cohorts
4. Analyses, interpretation is resource-intensive
5. Paucity of expertise in
    1. Analyzing and interpreting genomic data
    2. Functional analyses
    3. Integrative molecular pathogenesis

Current Top Priorities

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- Identify opportunities for genomic data for non-malignant outcomes
  - Ensure that the current-approved concepts are progressing in a timely fashion
  - Facilitate addressing relevant research questions and analyses of WES/ WGS data

Future Top Priorities

CCSS

- Develop integrated (clinical + genetic) risk prediction models that identify cancer survivors at highest risk for specific outcomes
- Develop interventions that target those at highest risk of complications – personalized/ targeted interventions
- Focus on pathogenesis of treatment-related complications
  - Functional studies stemming from the genomic leads
    - iPSC derived models
    - *In vitro* animal models
    - Utilization of somatic tissue (SMNs) integrated with germline variants/ mutations