Working Group Reports

Genetics

Smita Bhatia

Thursday, June 15, 2017

Membership

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

Level of Activity

- Weekly meetings
  - Wednesdays at 12:30 pm Central Time

Updates…

6/2017

Telomere homeostasis and thyroid cancer – Ancillary Study

MM Gramatges, TCCC

Clinical Cancer Research

Human Cancer Biology

See related article by Shay, p. 779

2014 Feb 15;20(4):904-11.

Telomere Content and Risk of Second Malignant Neoplasm in Survivors of Childhood Cancer: A Report from the Childhood Cancer Survivor Study

Maria M. Gramatges[1], Qi Liu[3], Yutaka Yasui[3], M. Fatih Okcu[1], Joseph P. Neglia[4], Louise C. Strong[2], Gregory T. Armstrong[5], Leslie L. Robison[5], and Smita Bhatia[6]

Telomere homeostasis and thyroid cancer – Ancillary Study

(PQB-1) R01 CA194473 (PI: MM Gramatges)

Defects in telomere maintenance associated with thyroid SMNs in childhood cancer survivors

Aim 1 Investigate the association between SNPs in telomere maintenance genes and thyroid SMN risk

Aim 2 Characterize rare genetic telomere maintenance defects in survivors with thyroid SMN (targeted DNA sequencing, functional studies)

Aim 3 Extend and improve upon the previously validated risk prediction model for thyroid SMN

R01 CA194473-02 Administrative Supplement (PI: MM Gramatges) 09/01/16-8/30/17
(PQB-1) Socioeconomic disparities, telomere maintenance, and thyroid second cancer risk

Major Goals: To expand the current study to investigate differences in socioeconomic (SE) factors in relation to telomere length as determined by leukocyte telomere content and sequencing (Aim 2), in order to improve upon the existing risk prediction model for thyroid SMN

Admin supplement included addition of a second control for each case as well as including SES data.

Second control identified in May 2017, SES data sent to PI.

TC analysis pending receipt of additional control samples, as well as any new thyroid SMN cases and matched control identified after the FU5 data freeze.

R01 CA194473 (PI: MM Gramatges) 09/01/15-8/30/19

Defects in telomere maintenance associated with thyroid SMNs in childhood cancer survivors

Aim 1 **Investigate association between SNPs in telomere maintenance genes and thyroid SMN risk**

SA1 leverages existing CCSS GWAS data to determine if SNPs distinguish survivors with thyroid SMN

- CCSS GWAS data sent to PI, PI awaiting identification of cases and controls to analyze data

Expand telomere content (TC) analysis from the initial CCSS study to include all thyroid SMN and matched non-SMN survivors,

- DNA from 64 case/control pairs sent to PI

R01 CA194473 (PI: MM Gramatges) 09/01/15-8/30/19;

Defects in telomere maintenance associated with thyroid SMNs in childhood cancer survivors

Aim 2 Characterize rare genetic telomere maintenance defects in survivors with thyroid SMN (targeted DNA sequencing, functional studies) rare/deleterious mutations in telomere maintenance genes enriched in thyroid ca

2.1 Perform targeted DNA sequencing of telomere maintenance genes in SA1 in CCSS subjects with thyroid SMN, 1:1 matched non-SMN survivors, and cancer naïve controls.

Admin supplement expanded this to WGS, but analysis on hold pending completion of Aim 2.3.

2.2 Functionally analyze in vitro ~30 rare missense mutations in the principal components of telomerase and compare the enrichment of all mutations both predicted and confirmed to be deleterious in thyroid SMN survivors with 1:1 matched non-SMN survivors and cancer naïve controls.

On hold pending completion of Aim 2.1.

2.3 Assess telomere maintenance capacity by measuring leukocyte subset absolute telomere length in thyroid SMN and 1:1 matched non-SMN survivors.

38 case control pairs sent to PI, submitted for telomere flow FISH. Recovery ~50% (poor viability of frozen lymphocytes); repeating analysis for failed cases/controls with additional vials if available.

Prelim data is promising; 30% of the 21 subjects with results have LTL ≤10th percentile.

Most notable difference is in naïve T cells, where TL is shorter among SMN survivors, suggesting premature senescence in this WBC population may be related to impaired cancer surveillance.

Of the 21 cases/controls with results, there are only 5 matched pairs - so the p value for this difference is NS (0.15)

R01 CA194473 (PI: MM Gramatges) 09/01/15-8/30/19;

Defects in telomere maintenance associated with thyroid SMNs in childhood cancer survivors

Aim 3 **Extend and improve upon the previously validated risk prediction model for thyroid SMN**

SA3 will extend a previously validated risk prediction model for thyroid SMN

Status *Dependent upon completion of SA1 and SA2*

Genetic Alterations in Second Malignant Neoplasms

Jean Nakamura, UCSF

Genetic Alterations in Second Malignant Neoplasms

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

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

Aim 3 **Sequence exons of 360 frequently mutated genes** in human cancers/genes in known cancer-relevant pathways.

Funding St. Baldrick's Foundation

Genetic Alterations in Second Malignant Neoplasms

Specimens Unstained, fixed sections of SMN samples (FFPE)/ paired normal samples (saliva)

Methods DNA isolated from FFPE SMN samples
SNP genotyping (Taqman)
Targeted exome sequencing

SMN type	Requested SMN tissue/ paired germline DNA	Received SMN tissue/ paired germline DNA
Breast	48/48	43/39
Meningioma	23/23	6/0
CNS	18/18	2/0
Sarcoma	10/10	4/0

Clinical Cancer Research

ıant neoplasms from pediatric cancer survivors

Amy Sherborne, Vincent Lavergne, Katharine Yu, Leah Lee, Philip Davidson, Tali Mazor, Ivan V. Smirnov, Andrew E Horvai, Mignon Loh, Steven G Dubois, Robert Goldsby, Joseph P. Neglia, Sue Hammond, Leslie L. Robison, Rosanna Wustrack, Joseph F Costello, Alice Nakamura, Kevin M. Shannon, Smita Bhatia, and Jean L Nakamura

2017 Apr 1;23(7):1852-1861

- Performed WES and RNASeq on radiation-induced sarcomas arising from 2 pediatric cancer survivors (UCSF)
  - WES revealed TP53 mutations involving p53's DNA binding domain in both index cases, one of which was also present in the germline.
  - Germline and somatic TP53 mutant variants were enriched in the transcriptomes for both sarcomas.
- Sanger sequencing performed to analyze germline TP53 in 37 pediatric cancer survivors with SMNs (CCSS)
  - Analysis of TP53 coding exons in germline specimens from the CCSS survivor cohort identified ten out of 37 evaluable patients (27%) harboring a germline TP53 variant.
- **Conclusions:** Identifying germline TP53 variants at the time a primary cancer is diagnosed may identify patients at high risk for SMN development, who could benefit from modified therapeutic strategies and/or intensive post-treatment monitoring

Genetic Alterations in Second Malignant Neoplasms

On-going work: WES of the CCSS FFPE SMN samples. Isolated genomic DNA and have tested exome library preparation protocols on select samples

Barriers: Uneven quality of the FFPE tissue samples poses challenges for sequencing.

Timeline: Anticipate that in the next year or so, will have optimized sequencing protocols for FFPE tissues. Contingent on funding, exome sequencing will be carried out.

Susceptibility genes for radiation-induced breast cancer after HL

van Leeuwen/ Netherlands

Radiation-induced breast cancer after HL – germline variants

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

Design Case-control study design

Cases: Caucasians with HL (age <40y) + secondary breast cancer (>8y from HL)

Controls: Caucasians with HL (age <40y) + free of breast cancer until date of inclusion

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

2nd comparison with available data on young BC without HL

Platform Illumina iSelect 200,000 targeted SNP chip

Status Cases (n=335)/ controls (n=502) identified
(CCSS [69/56]/ UK [153/275]/ NL [117/177])

Examined 211,155 SNPs genotyped using a Illumina custom array (iCOGs)

Compared the breast cancer cases with matched sample of 4671 sporadic breast cancer cases for whom iCOGS results were available already

Several interesting hits tested in the case-control design

Manuscript under preparation

Genetic Susceptibility to Obesity after Childhood ALL

Kala Kamdar, Philip Lupo

Baylor

Genetic Susceptibility to Obesity after Childhood ALL

Aims

Using GWAS, determine relation between treatment-related obesity and genetic polymorphisms (SNPS and CNVs) after ALL

Replicate top SNPs, CNVs from discovery phase in an independent sample of ALL survivors from TCH

Funding

Leukemia Lymphoma Society

Status

GWAS analysis complete (as part of NCI GWAS initiative) – Discovery (n=1,184)

Analyzed directly-genotyped SNPs (i.e. did not impute)

No genome-wide level hits in overall group

Adjusted for age at diagnosis, age at date of assessment, gender, CRT, genetic ancestry

Stronger effects in CRT group (i.e., one locus was genome-wide significant $p < 5x10^{8}$)

Received imputed data from collaborators at NCI and are re-running discovery analysis

Replication ongoing in 538 ALL survivors from TCH

received genotype data from NCI, updating obesity phenotype

Neurofibromatosis and subsequent malignancies

PI **Smita Bhatia, UAB**

Funding: P50-CA196519-01

Neurofibromatosis and subsequent malignancies

Aim 1 Describe the risk of SMNs in individuals with NF1

Resources: CCSS and NF1 Registries at CHOP and UAB

*i) children with NF1 and primary neoplasia (**NF1⁺cohort: CCSS, CHOP, UAB**);*

*ii) children with neoplasia, but without NF1 (**non-NF1cohort: CCSS**)*

Aim 1.1 *Compare SMN risk in the **NF1⁺cohort** with SMN risk in **non-NF1 cohort***

Aim 1.2 *Compare SMN risk in **NF1⁺cohort** exposed and not exposed to radiation and/or chemotherapy*

Status: CCSS – received and analyzed data/ data at CHOP currently being abstracted/ UAB data abstracted

Aim 2 Identify genetic alterations associated with radiation-induced tumorigenesis in individuals with NF1 *Targeted exome sequencing of paired SMN/ germline tissue from individuals with NF1 to identify tumor-promoting pathways; characterize mutational landscape*

Aim 3 Validate biologic importance of candidate pathways in radiation-induced tumorigenesis; determine whether radiotherapy promotes development of MPNSTs.

mouse cell lines; mouse models

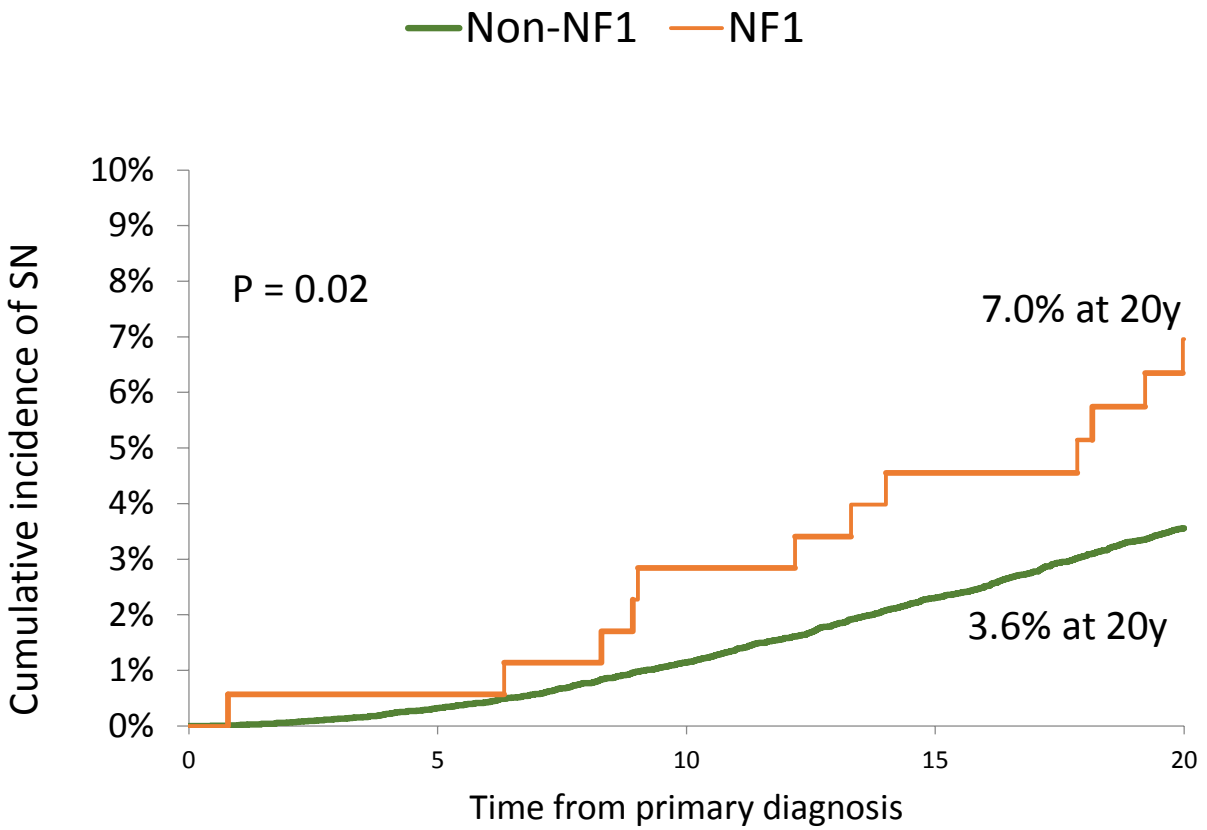
Aim 1.1 Are NF1 individuals with a primary neoplasm at increased risk of developing SNs compared with non-NF1 individuals with a primary neoplasm?

Demographic and Clinical Characteristics of the CCSS population	Overall (n=24,357)	Non-NF1 (n=24,181)	NF1+ (n=176)	P-value
Age at diagnosis (mean [SD])	8.2y±5.8	8.2y±5.8	6.6y±5.1	<0.0001
SN event by 20y				
No event	13,068 (53.7%)	13,007 (53.8%)	61 (34.7%)	<0.0001
SN	1,187 (4.9%)	1,174 (4.9%)	13 (7.4%)	
Death	10,102 (41.4%)	10,000 (41.4%)	102 (58%)	
Race				
NHW	19,474 (80%)	19,324 (79.9%)	150 (85.2%)	0.07
Other	4,883 (20.1%)	4,857 (20.1%)	26 (14.8%)	
Primary Diagnosis				
CNS	4,323 (17.8%)	4,176 (17.3%)	147 (83.5%)	<0.0001
Other	20,0034 (82.3%)	20,005 (82.7%)	29 (16.5%)	

Aim 1.1 Are NF1 individuals with a primary neoplasm at increased risk of developing SNs compared with non-NF1 individuals with a primary neoplasm?

Demographic and Clinical Characteristics of the CCSS population	Overall (n=24,357)	Non-NF1 (n=24,181)	NF1+ (n=176)	P-value
Anthracyclines				
Yes	10,553 (43.3%)	10,539 (43.6%)	14 (8%)	<0.0001
Platinum				
Yes	2,553 (10.5%)	2,511 (10.4%)	42 (23.9%)	<0.0001
Alkylating agents				
Yes	12,015 (49.3%)	11,985 (49.6%)	30 (17.1%)	<0.0001
Any radiation				
Yes	12,732 (52.3%)	12,668 (52.4%)	64 (36.4%)	<0.0001

Cumulative Incidence of SNs by NF1 status (excluding basal cell carcinoma)



SN type in NF1 patients	Number
Oxyphilic adenoma	N=1
Serous surface papillary carcinoma	N=1
Malignant fibrous histiocytoma	N=1
Ependymoma	N=1
Astrocytoma	N=3
Glioblastoma	N=2
MPNST	N=1
Neurilemmoma	N=1

Risk of SNs in NF1 patients with primary neoplasia c/w those without NF1 and primary neoplasia – multivariable analysis

Variables	HR	95% CI	P-value
NF1 status			
No NF1	1.0		
NF1+	**2.7**	**1.4-4.6**	**0.001**
Age at diagnosis	1.05	1.04-1.06	<0.0001
Race			
NHW	1.0		
Other	1.2	0.9-1.4	0.09
Anthracyclines	0.8	0.7-0.9	0.003
Alkylating agents	1.4	1.2-1.6	<0.001
Radiation	2.9	2.1-2.8	<0.0001

Proportional Subdistribution Hazards Regression for Survival Analyses with Competing risks

Aim 1.2 Are NF1 individuals with a primary neoplasm and exposure to radiation and/or chemotherapy at increased risk of developing SMNs as compared with NF1 individuals not exposed to radiation or chemotherapy?

- For this aim we will include only NF1 patients with primary neoplasia – with and without SN
- In order to maximize power, we will include the sample from CCSS (n=176; 13 with SN), UAB (n=58) and CHOP (n= 114)
- We have therapeutic data on the cohort from CCSS and UAB
- Therapeutic abstraction from CHOP underway.

Molecular characterization of therapy-related pediatric high-grade gliomas

- AOI approved
- Lucas/ SJCRH

Status: samples have been identified – study to start soon

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

GWAS resource for Genetic Investigation

- **Collaboration with NCI Division of Cancer Epidemiology and Genetics**
  - Genotyping platform
    - Illumina® HumanOmni5-Quad BeadChip (~4.1 million SNPs)
- **Number of samples genotyped**
  - 5324 (European descent); 415 (non-European ancestry)
- **Aims**
  - Identify genetic variants associated with subsequent neoplasms

GWAS cohort – entire cohort

	Cohort	Cases	Controls
Any SN	5324	877	4447
Any RT-related	5242	795	4447
BCC	4809	362	4447
Breast	2378	178	2200
Meningioma	4572	125	4447
Thyroid	4537	90	4447
Sarcoma	4505	58	4447

Analyses in progress

JNCI, in press

Analyses in progress

Exome sequencing to discover genetic variants that predispose childhood cancer survivors to the development of subsequent neoplasms (CCSS/NCI)

Current status

- Whole exome sequencing (WES)
  - NCI Cancer Genomics Research Laboratory
  - Illumina HiSeq, NimbleGen v3.0+UTR sequence capture
  - Mean 40x coverage
  - Bioinformatics: In-house pipeline with machine learning algorithm to combine results from multiple variant callers

Post-build quality control evaluation

1. Identify genetic variants associated with the development of **subsequent neoplasms** among childhood cancer survivors
2. Identify genetic variants associated with the risk of **childhood cancer**
3. Develop a **resource** of genetic data.

Requests for replication…

- **Genetic susceptibility to stroke (Discovery – SJLIFE)**

  K Krull

- **Cisplatin-induced hearing loss (discovery – CHLA)**

  D Freyer

- **Late effects prediction using clinical phenotypes and whole genome sequencing (discovery – SJLIFE)**

  Y Yasui

- **Genetic susceptibility to premature menopause (discovery – SJLIFE)**

  Y Yasui

- **Genetic Susceptibility to anthracycline-related CHF (Discovery COG)**

  S Bhatia

Genetics Working Group

Current Priorities

Vision for next 5 years

Genetics Working Group

Current priorities (next 12 to 24 months)

1. Facilitate completion/ publication of studies currently underway
2. GWAS
    1. Facilitate publication of GWAS/SMN studies
    2. Encourage/ facilitate use of GWAS data for non-SMN outcomes

Request for Proposals

use of GWAS data and corresponding non-malignant outcomes data

Request for Proposals

- Collaboration with CCSS and NCI investigators in the **use of GWAS data and corresponding outcomes-related data** to address innovative research questions relating to potential genetic contributions to risk for treatment-related outcomes.
- Requests are being accepted on an ongoing basis
- Requests will be reviewed three times per year, in February, June and October
  - cut-off for receipt of applications Feb1st, June 1st, Oct 1st

Request for Proposals

- All GWAS data is being posted on dbGap with following covariates
  - Age at diagnosis, Sex, Race/ ethnicity
  - Primary cancer diagnosis, Vital status
  - Date of sample procurement
  - Date of genotyping
- Researchers desiring additional covariates must submit a full application.
  - All projects will be viewed as a **collaboration with one or more investigators from the CCSS and NCI** being part of the research team
  - All **linkage of genotype and phenotype data** (beyond that posted on dbGap) will only be **carried out by the CCSS Data and Statistics Center**
- Data analyses may be performed by qualified individuals proposing the research, by CCSS biostatisticians, or both
  - All analyses and results must be vetted by CCSS biostatisticians prior to submission for publication

Request for Proposals

- Investigators proposing to use GWAS data will comply with the following (by signing Data Use Certification)
  - Limiting use of data to the project described in Application for Data Access
  - Not distributing data beyond those directly engaged in the analysis outlined in the Application for Data Access
  - Not attempting to identify or contact study participants from whom phenotype/genotype data were collected
  - Adhering to policies on timeframe for publications stemming from data
  - Providing annual detailed reports
  - Institutional documentation that CCSS material transfer agreement is acceptable
- Any violation of these policies can result in withdrawal of the project with return of CCSS data

Request for Proposals

- PI proposing to use GWAS data must submit application for Data Access to Greg Armstrong
  - Specific Aims
  - Background/significance of research question
  - Requested data elements
  - Approach (plans for validation, functional studies)
  - Statistical considerations
  - Timeline

  5 pages
  - Cited Literature

  1 page
  - Investigative team and qualifications
  - NIH Biosketch for each investigator
  - Available resources/funding plans

Request for Proposals – Review Process

- **Applications will undergo a two-stage review process**
  - The initial review will be conducted by the CCSS Genetics Data Access Committee
  - Those applications approved by the Genetics Data Access Committee will be submitted to the CCSS Research and Publications Committee for review and comment

Request for Proposals – Review

- **Reviewed applications will be categorized as**
  - approved
  - approved with stipulations
  - not approved, but a revised application may be reconsidered
  - disapproved, no future consideration.

Request for Proposals – Post Approval Process

- The PI and his/her research team will work together with CCSS collaborators assigned to the project to facilitate construction of the analytic data set, establish a task-specific timeline, agree upon a tentative list of target journals, and authorship.
- A written progress report will be required annually until the project has been completed (i.e. publication).
  - CCSS reserves the right to withdraw approval for any project that fails to demonstrate adequate progress (i.e., if the project is delinquent by >12 months after the designated timeline).
- Review and approval of results by the CCSS Genetics Working Group Chair and the assigned CCSS biostatistician is required prior to any presentation or publication of the data from the project.

Request for Proposals

Data Access Committee

- Greg Armstrong
- Les Robison
- Yutaka Yasui
- Lindsay Morton
- Joshua Sampson
- Wendy Leisenring
- Smita Bhatia

	Any		Maximum grade				
	No	Yes	1	2	3	4	5
SN	4483	841	__	327	149	319	46
Loss of hearing	4592	732	417	9	274	32	__
Cataract	5099	225	172	__	53	__	__
Blindness	5088	236	14	24	156	42	__
Hypothyroidism	4577	747	121	626	__	__	__
Diabetes	5135	189	59	74	56	__	__
Gonadal dysfunction	5078	119	30	1	215	__	__
Heart attack	5203	121	__	__	35	83	3
CHF	5096	228	8	83	121	11	5
Hypertension	4539	785	185	598	1	__	1
Stroke	5161	163	4	2	2	152	3
Dyslipidemia	4636	688	146	542	__	__	__
Dialysis	5259	65	22	__	2	41	__

June 1, 2017 RFP deadline

6 applications submitted – 5 approved

Genetics Working Group

Vision for next 5 years

1. **GWAS**
    1. Facilitate approval of SMN concepts in the queue pending primary GWAS analysis
    2. Encourage/ facilitate use of GWAS data for non-SMN outcomes

2. **Whole Exome Sequencing**
    1. Understand contribution of mutations in the exome on risk of SMNs and chronic health conditions in childhood cancer survivors

3. **Somatic mutational landscape/ pathogenesis of SMNs**
    1. Pair with sequencing in SMN tissue to understand the pathogenesis of SMNs
        1. Aggressive banking of SMN tissue – frozen if possible
        2. Sequencing of paired tissue/germline DNA

Distribution of Biologic Material Available for Invasive SMN Cases as of 05/2017

SMN	Total case reported	Total with path reports	# with host tissue			Host tissue (any kind) and MRAF data	Total with MRAF data	Total with SMN tissue	Number of Cases with Tissue Type			
			Buccal cells	Oragene	Blood				H&E Slides	Unstained Slides	Scrolls	Blocks
Breast	447	309	222	230	204	312	430	159	144	102	28	49
Meningioma*	336	179	175	188	136	241	313	52	50	42	12	19
Other CNS	124	66	26	24	16	34	120	17	12	15	1	2
Thyroid	262	140	106	150	122	193	257	76	73	64	24	21
Sarcoma	108	75	34	32	28	48	100	20	17	19	4	3
Leukemia	54	24	9	11	7	13	48	5	5	4	0	1
Bone	54	38	11	9	10	17	46	1	1	1	0	1
Melanoma	67	37	26	34	26	45	65	10	9	9	2	1
Lymphoma	56	34	17	21	17	27	52	8	7	6	1	2
Renal Cell	43	25	19	22	13	24	40	4	4	4	0	1
Other Ca	212	125	84	90	61	120	205	45	44	31	3	15
NMSC*	2290	1626	1323	1274	513	1603	2167	5	5	3	0	1
All Other	59	21	21	19	12	27	54	8	7	5	0	1
TOTALS	4,112	2,699	2,073	2,104	1,165	2,704	3,897	410	378	305	75	117

Questions?