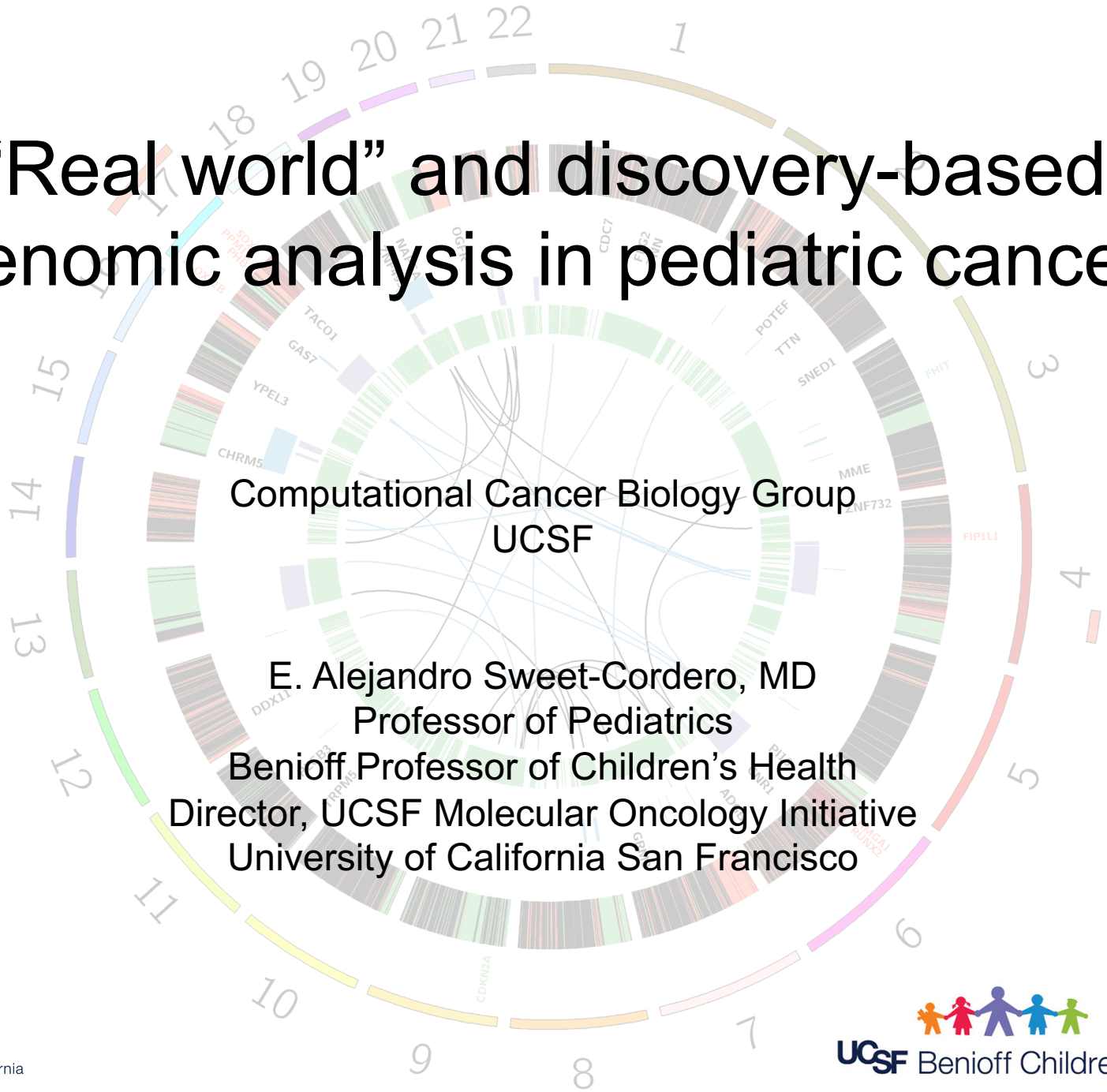
“Real world” and discovery-based genomic analysis in pediatric cancer



Computational Cancer Biology Group
UCSF

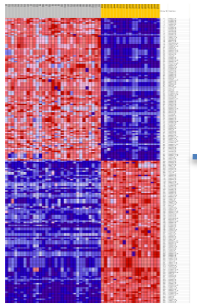
E. Alejandro Sweet-Cordero, MD
Professor of Pediatrics
Benioff Professor of Children’s Health
Director, UCSF Molecular Oncology Initiative
University of California San Francisco

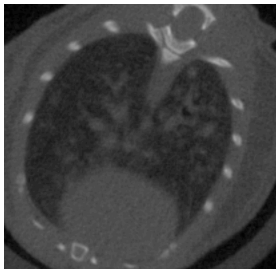
UCSF
University of California
San Francisco

UCSF Benioff Children’s Hospitals

Sweet-Cordero Lab (Rock Hall, MB)

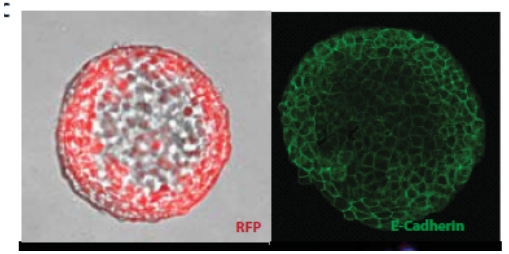
Functional Genomics of Tumor Progression, Metastasis, and Therapy Response in Cancer





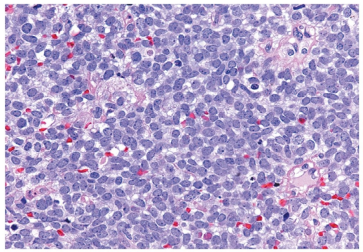
Functional genomics of oncogenic Kras

Vicent et al Cancer Research, 2012
Valencia et al JCI 2020
Gwinn et al, Cancer Cell 2018
Kelly, Kostyrko, Han, Cancer Discovery 2020



Tumor heterogeneity and therapy response in lung cancer

Zheng et al Cancer Cell 2013
Vicent et al Cancer Research 2013
Kim and Marquez et al Nature Medicine, 2019

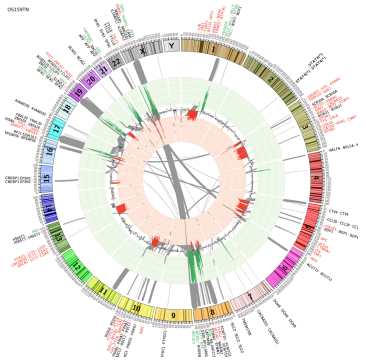


Molecular pathogenesis of Ewing Sarcoma

Marques et al, JCI 2014

"Real world" application of genomics to advanced pediatric/AYA cancer care

Vaske et al, JAMA 2019
Levinson et al, JCO Precision Oncology, 2020



Genomic instability and metastasis of osteosarcoma

Sayles et al, Cancer Discovery 2018

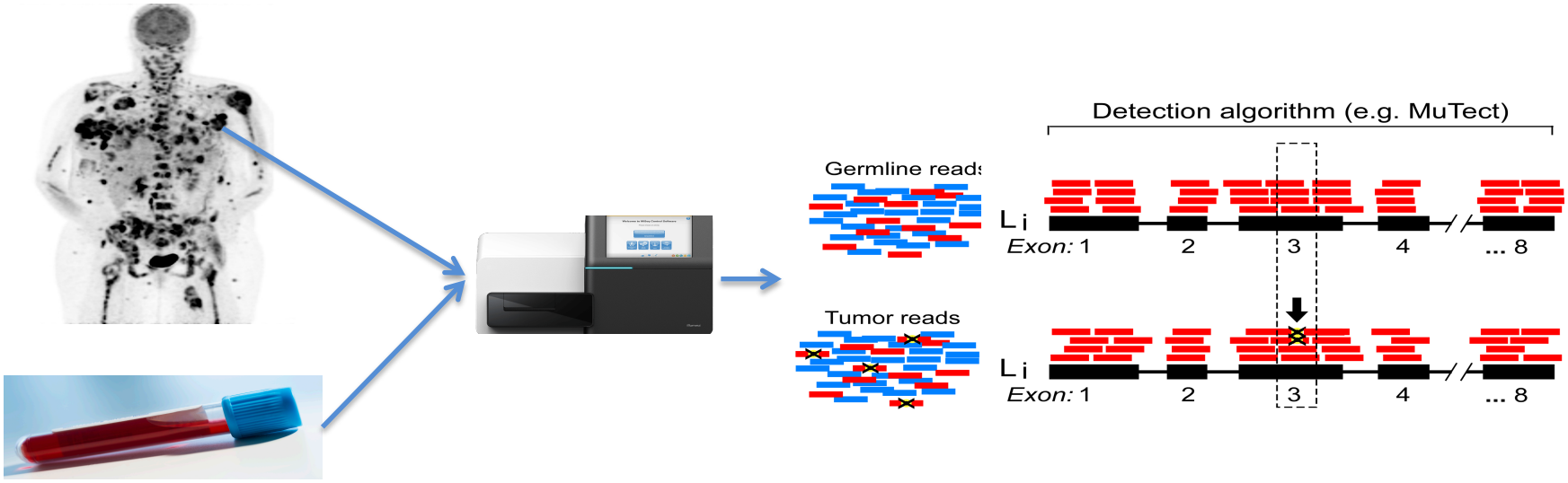
Outline

- Overview of UCSF clinical sequencing efforts
- Overview of UCSF integrative genomics efforts

Current state of precision cancer medicine in pediatric oncology

- Many clinical trials already incorporate genomic biomarkers.
- Idea of assigning therapy based primarily on presence of biomarkers (as opposed to histology) still relatively untested.
- Early studies focused on feasibility
- Current large Pediatric Match study underway.
- A key limitation is access to drugs and difficulty of designing combination drug studies.
- Research to Accelerate Cures and Equity (**RACE**) act improves access as it requires all drug companies to have a pediatric development plan.

UCSF500: NGS assay for germline and tumor analysis



- Test developed in house with consultation of both adult **AND** pediatric oncologists.
- DNA is extracted from FFPE for tumor and either blood or saliva for germline.
- Currently uses DNA only, RNA assay in development

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UCSF-HDFCC Molecular Oncology Initiative

Serving patients and providers

- Provide state-of-the-art, evidence-based recommendations to help guide interpretation of molecular testing performed in cancer patients at UCSF (Molecular Tumor Board).

- Develop new methods for measuring utility and feasibility of precision medicine in all patients.

Learn from our patients to help future patients

Data sharing integration & innovation:

- Drive innovation in data sharing and data analysis to improve delivery of precision cancer medicine.

- *Integration of genomics with the medical record*

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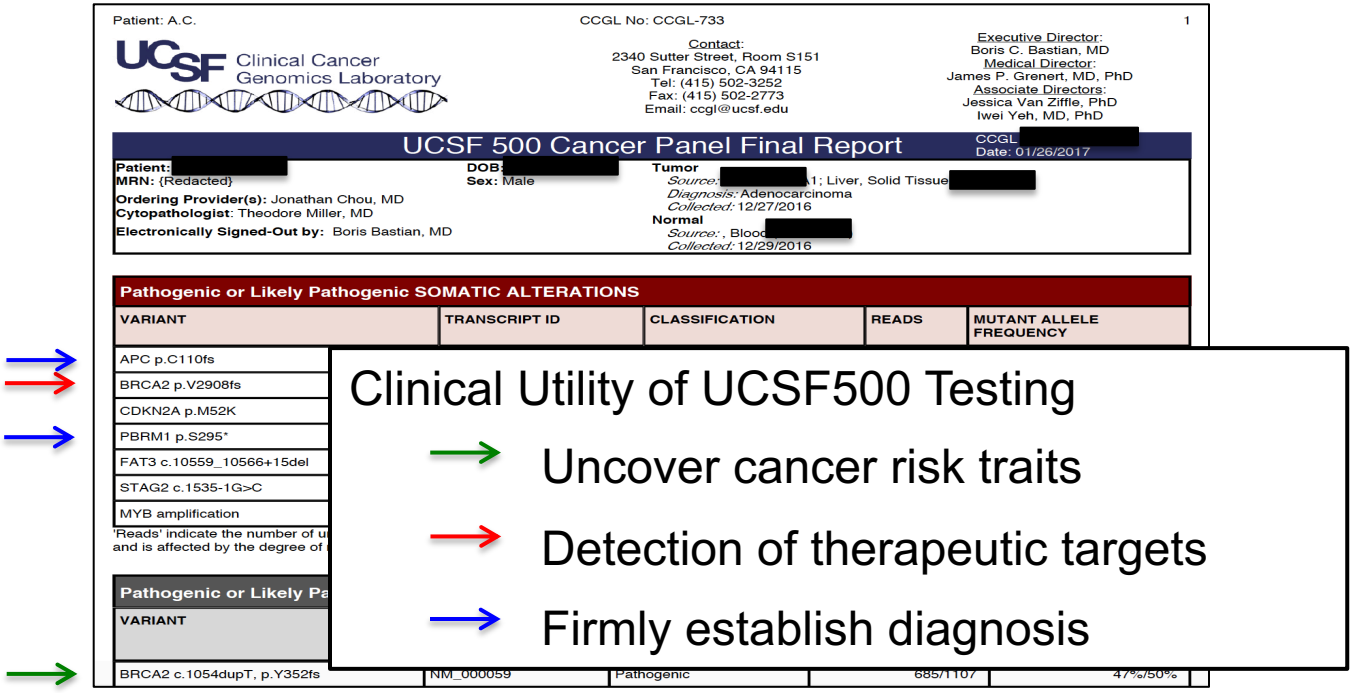
UCSF500 technical specifications

- Complete coverage of exons for 479 genes
- Selected tiling of introns for 47 genes (fusion detection).
- Foot print size ~4.78 (V4) and 2.9 (V3)
- Probes across genome to report copy number change
- Sequence to 500X depth
- DNAnexus used as bioinformatic platform
- TAT currently average of ~14 days
- Clinical report generated and given to clinician

UCSF500 clinical report



Patient: A.C. CCGL No: CCGL-733 1

UCSF Clinical Cancer Genomics Laboratory

Contact:
2340 Sutter Street, Room S151
San Francisco, CA 94115
Tel: (415) 502-3252
Fax: (415) 502-2773
Email: ccgl@ucsf.edu

Executive Director:
Boris C. Bastian, MD
Medical Director:
James P. Grenert, MD, PhD
Associate Directors:
Jessica Van Ziffle, PhD
Iwei Yeh, MD, PhD

UCSF 500 Cancer Panel Final Report

CCGL [redacted]
Date: 01/26/2017

Patient: [redacted]
MRN: {Redacted}
Ordering Provider(s): Jonathan Chou, MD
Cytopathologist: Theodore Miller, MD
Electronically Signed-Out by: Boris Bastian, MD

DOB: [redacted]
Sex: Male

Tumor
Source: [redacted]1; Liver, Solid Tissue [redacted]
Diagnosis: Adenocarcinoma
Collected: 12/27/2016

Normal
Source: , Blood [redacted]
Collected: 12/29/2016

Pathogenic or Likely Pathogenic SOMATIC ALTERATIONS

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS	MUTANT ALLELE FREQUENCY
APC p.C110fs				
BRCA2 p.V2908fs				
CDKN2A p.M52K				
PBRM1 p.S295*				
FAT3 c.10559_10566+15del				
STAG2 c.1535-1G>C				
MYB amplification				

'Reads' indicate the number of u
and is affected by the degree of

Pathogenic or Likely Pa

VARIANT				
BRCA2 c.1054dupT, p.Y352fs	NM_000059	Pathogenic	685/1107	47%/50%

Clinical Utility of UCSF500 Testing

- Uncover cancer risk traits
- Detection of therapeutic targets
- Firmly establish diagnosis

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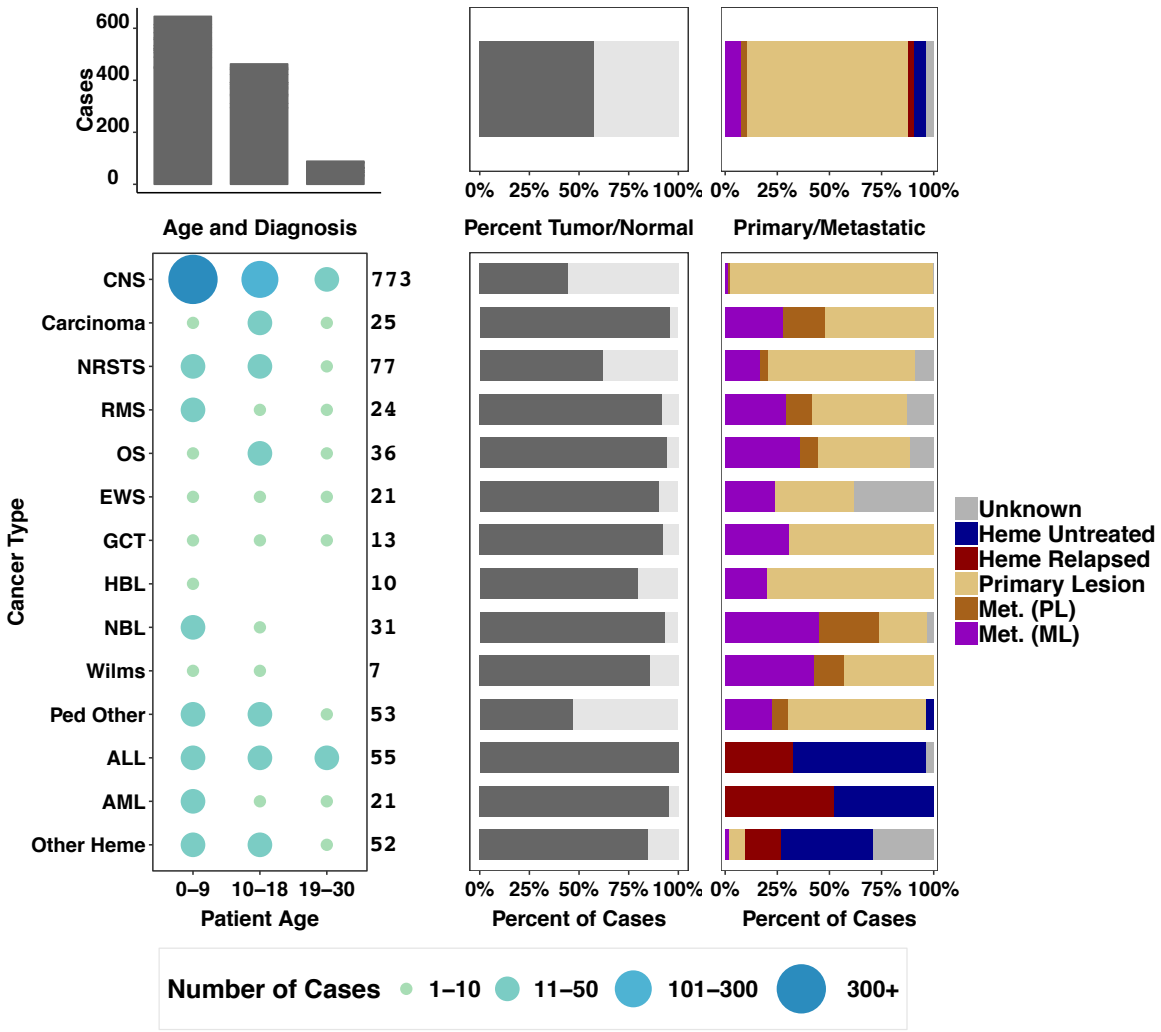
UCSF500 pediatric cases: Age and Diagnostic Distribution



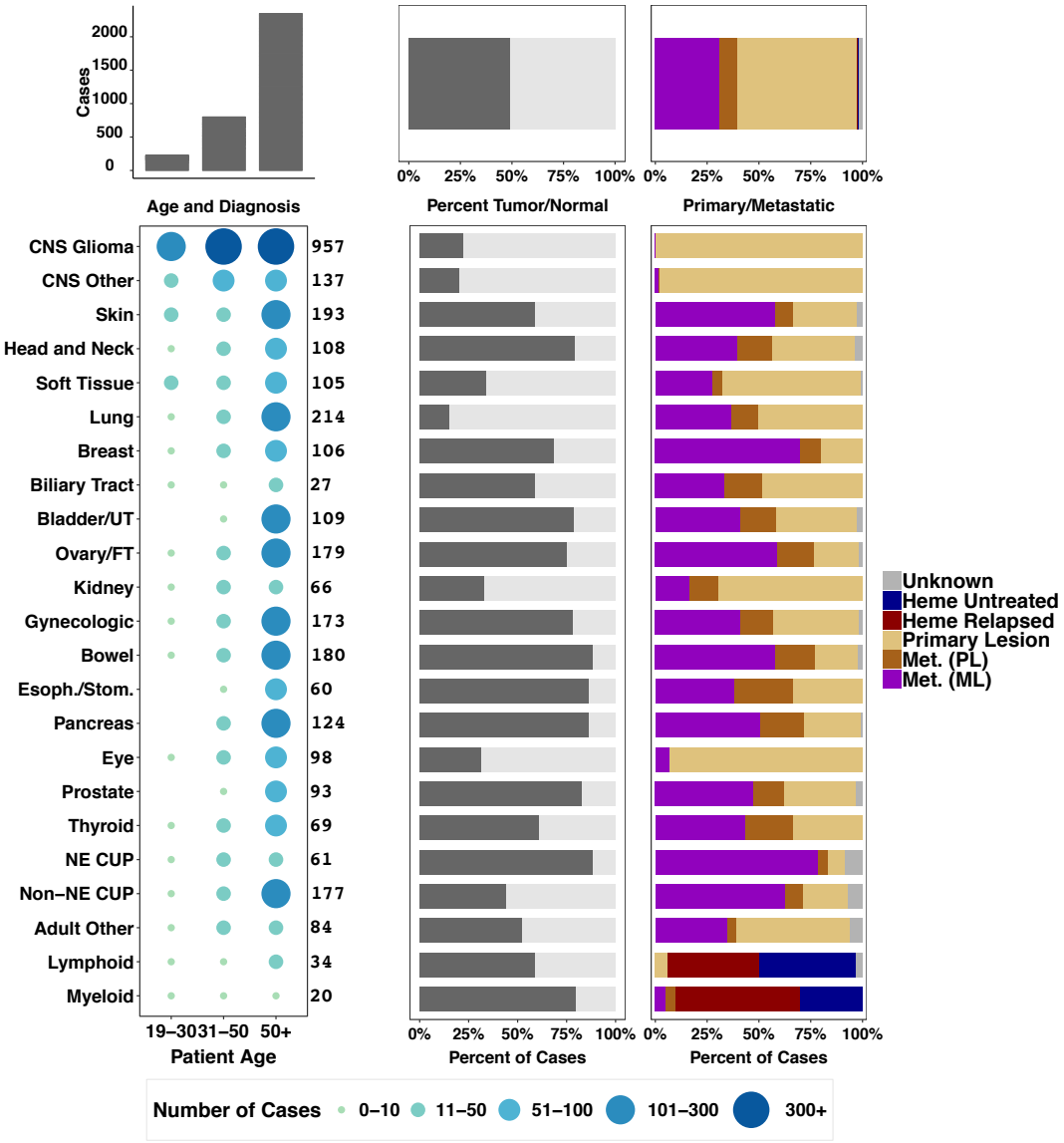
1198 pediatric/AYA cases:

*64% CNS

*26% extra cranial solid tumors

*10% Heme malignancies

UCSF500 adult cases

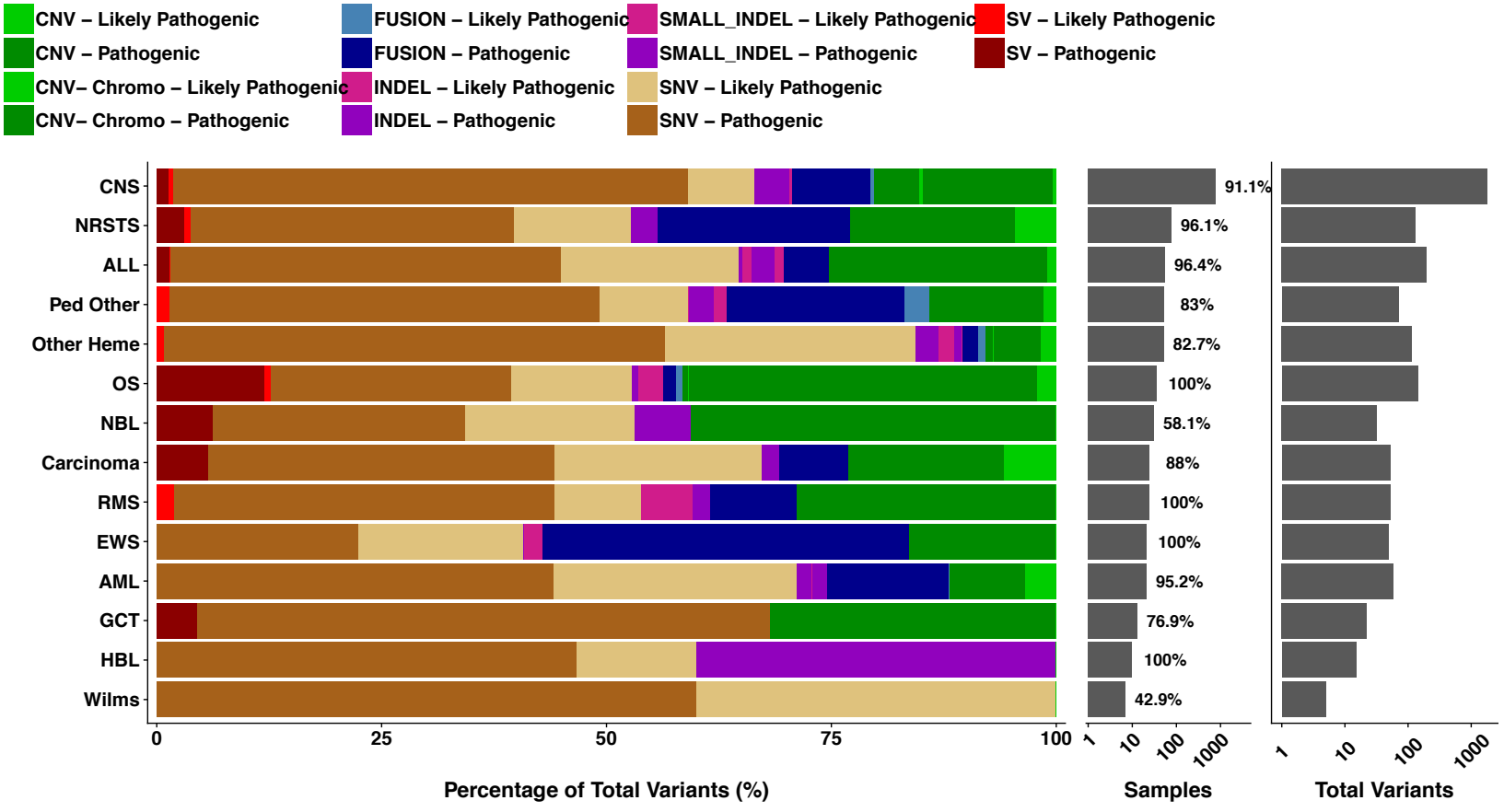


-3,374 sequenced cases

-~49% T/N

~1/3 neuro-onc

Distribution of pathologic/likely pathologic alterations by type and tumor site-pediatric cases.



- Wide variability in most common alteration seen between subtypes.
  - Fusion is single most common alteration in EWS (seen in all cases).
  - Number of distinct variants varies widely (CNS highest, Wilms lowest)

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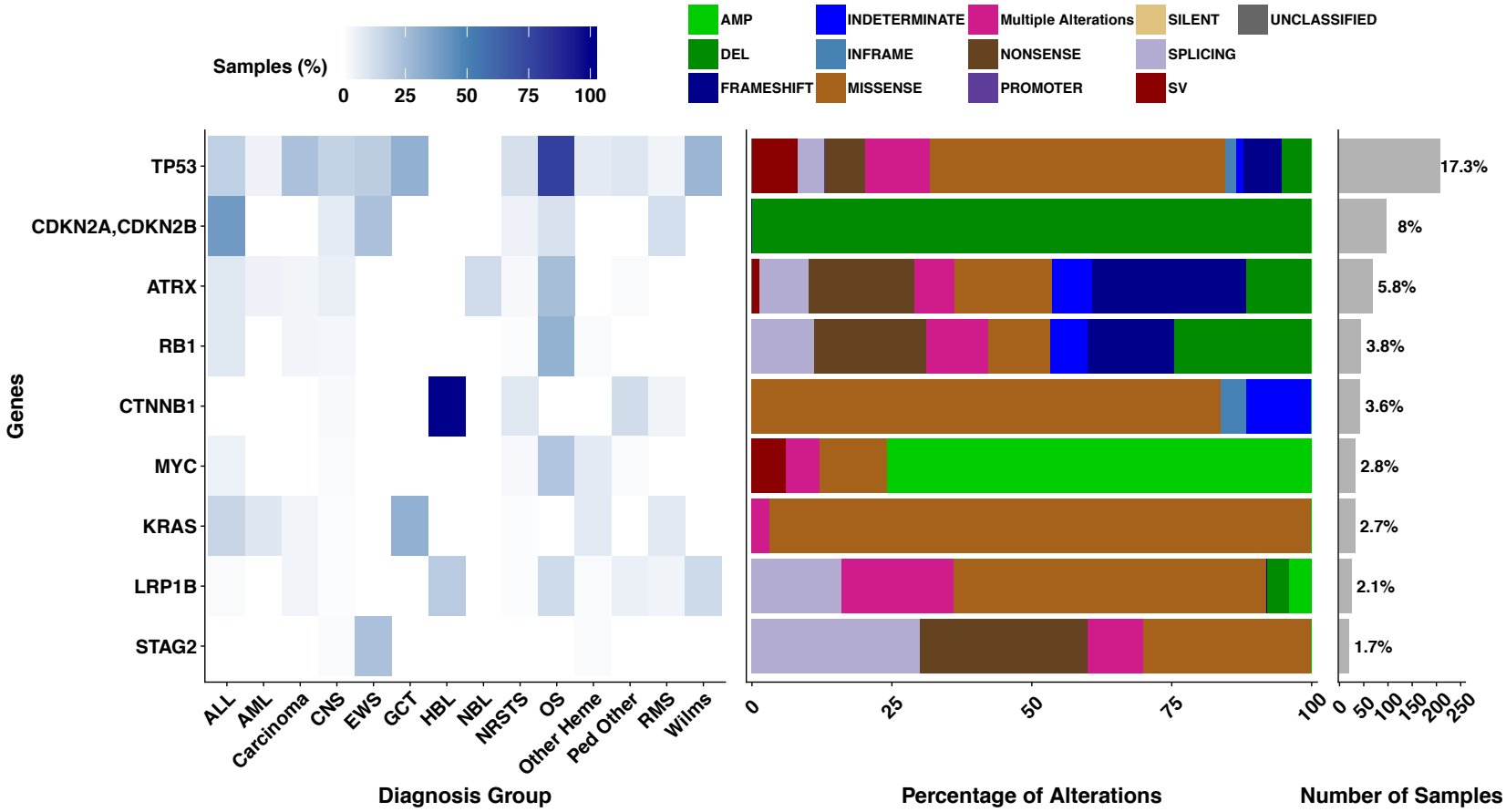
UCSF500: pediatric somatic common alterations-pediatric



- None of top 9 alterations are currently druggable (with exception of small fraction of KRAS)

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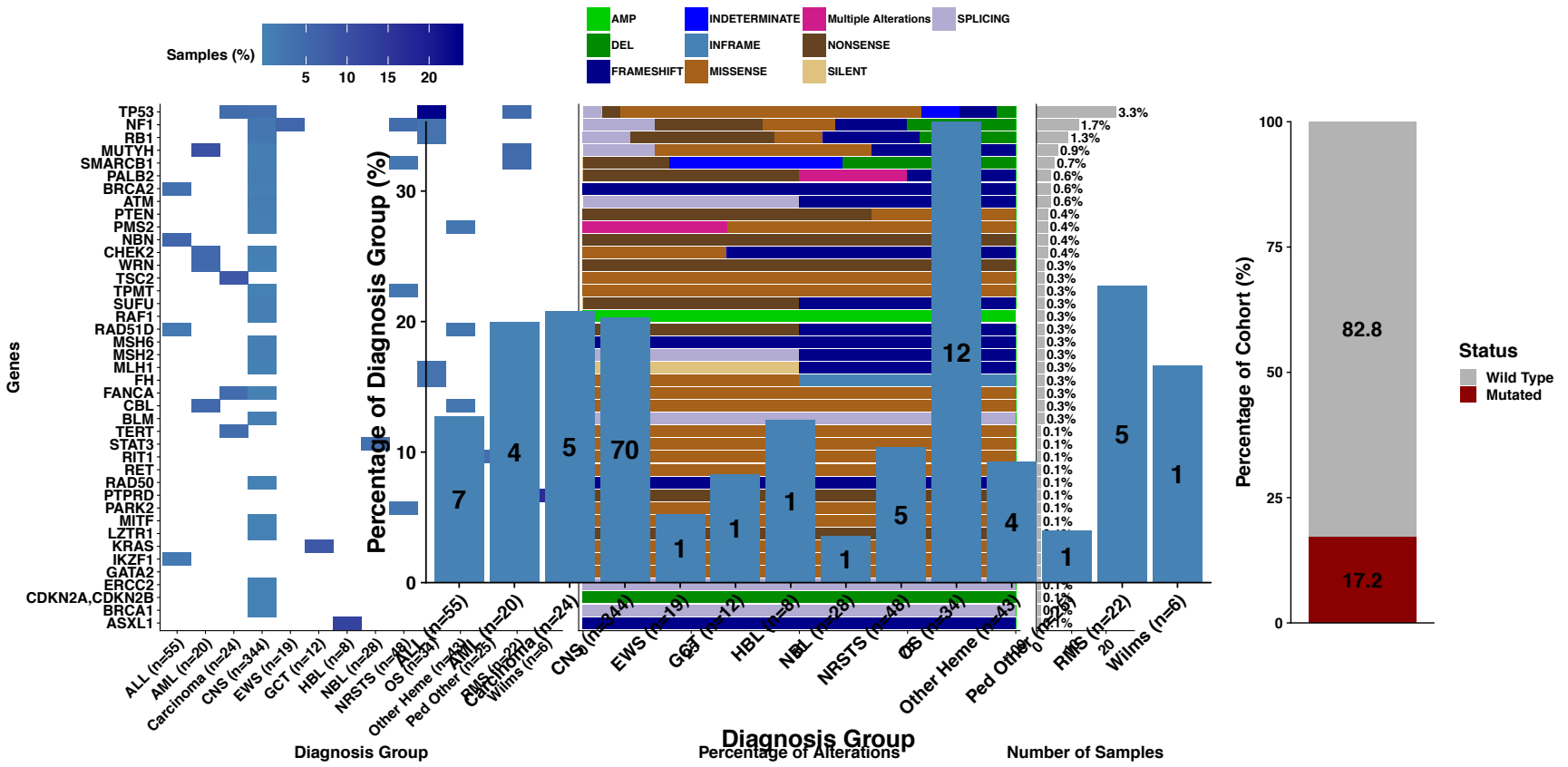
UCSF500: pediatric germline alterations



- Li-Fraumeni (p53) and NF most common germline alterations
- Some alterations are specific to disease subtypes (RAD51D and IKZF1 in heme malignancies).
- Some not previously well characterized (ASXL1 in HB)
- 17.2% of patients had a germline predisposition.

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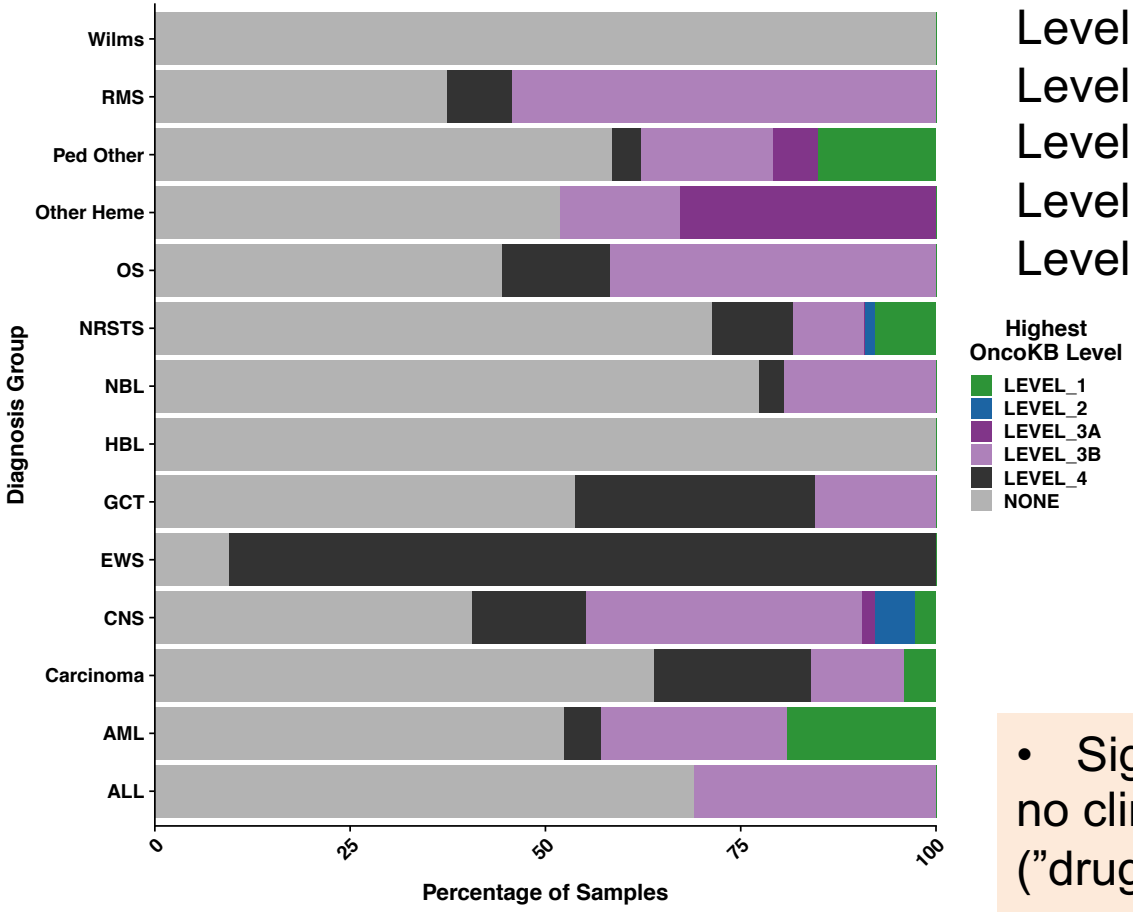
UCSF 500: Actionability in pediatrics

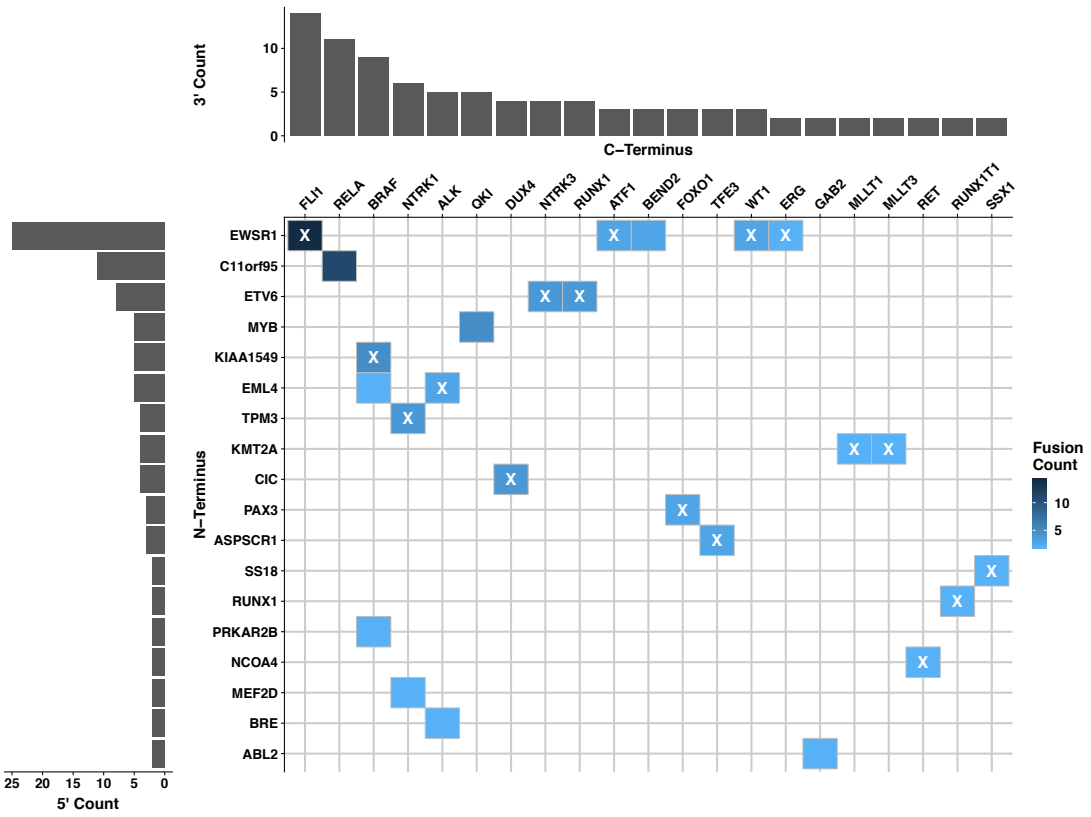


Level 1-FDA approved
Level 2A-standard of care/disease match
Level 2B- 2A-standard of care/other
Level 3A-clinical evidence/match
Level 3B-clinical evidence/other
Level 4-preclinical

- Significant percentage of cases have no clinical evidence for actionability (”druggability”)

- Most is level 3B, only small fraction Level 1/2

UCSF500-Fusion identification using a DNA panel



- Most fusions seen are rare (one patient)
- A few have recurrent 5' partner (EWSR1)
- A few have recurrent 3' partner (BRAF, ALK).
- Many are novel:
  - EWSR1-BEND1
  - MEFD2-NTRKI

-some fusions likely not seen because no RNA analysis

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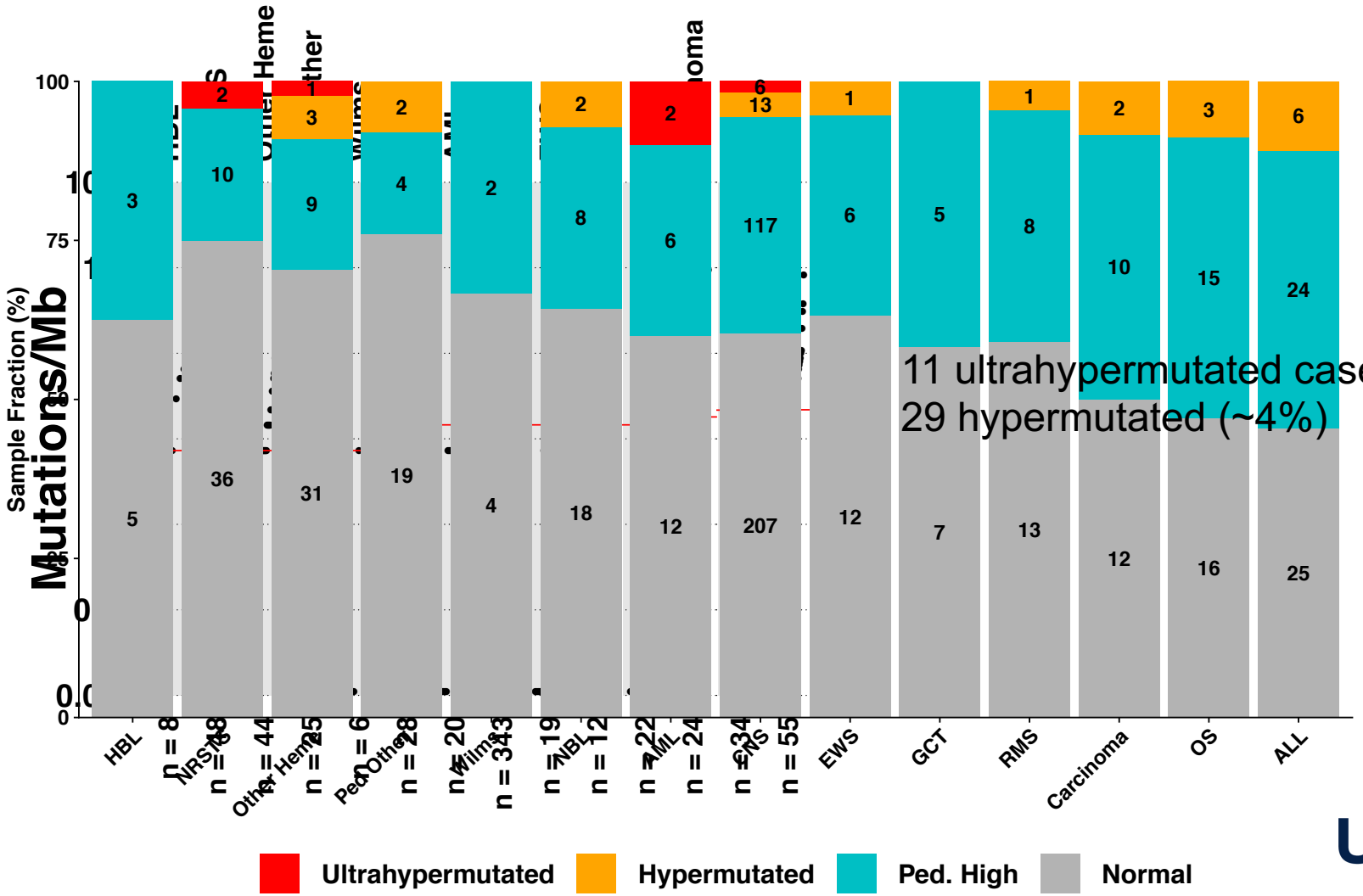
Tumor Mutational Burden (TMB) and other "second order" genomic alterations.

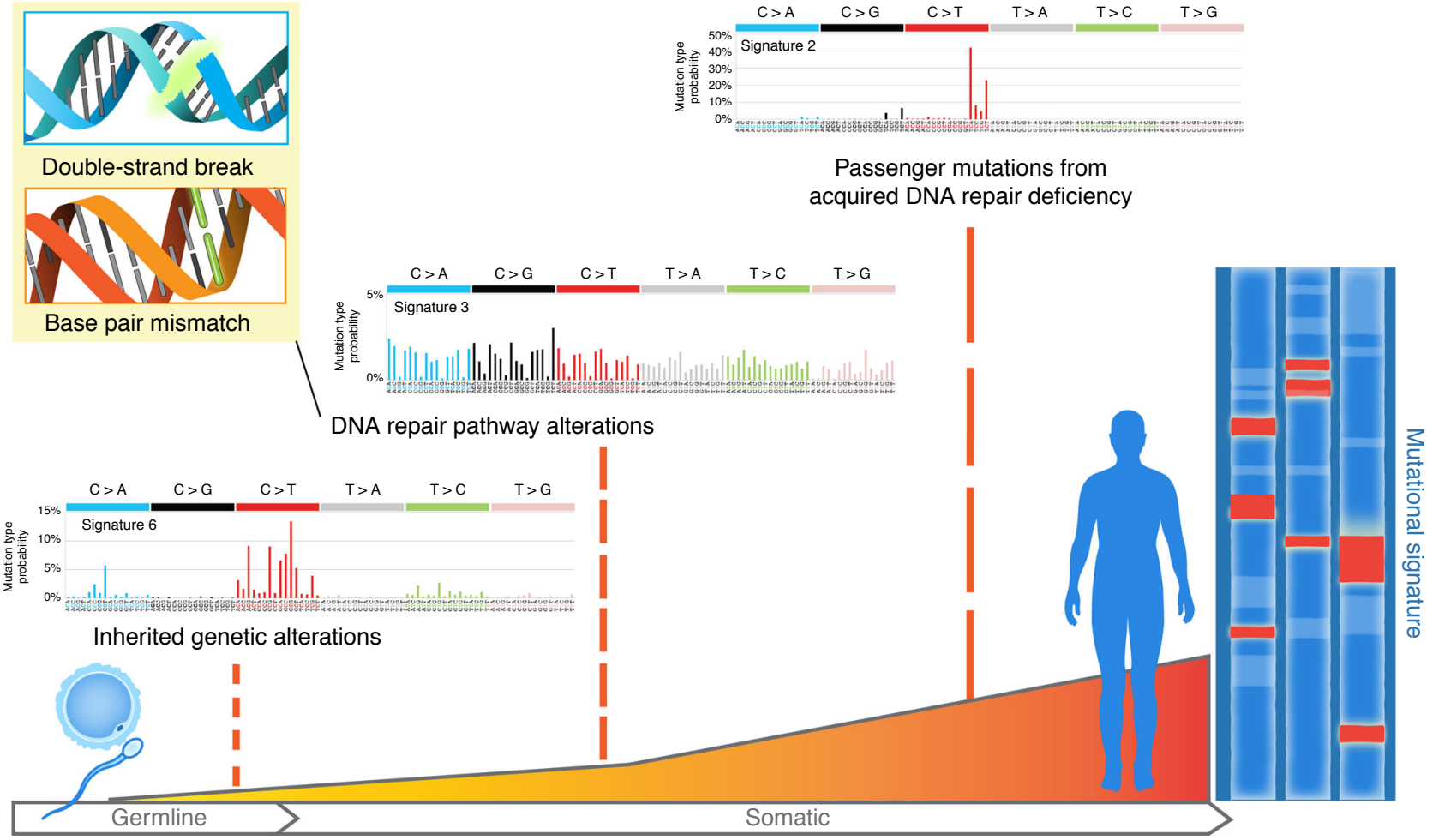
- In addition to finding specific mutations, we can also use sequencing to learn about overall features of the cancer genome:
  - Tumor mutational burden (TMB) is an emerging genomic biomarker in cancer
  - May serve as a proxy for cancer cell neo-antigens that could be detected by the immune system as foreign.
  - High TMB can be due to genetic factors (Mismatch repair defects, etc) or prior therapy (radiation, specific chemotherapeutic agents)
  - Mutational signatures are another genomic alteration that may indicate important aspects of etiology (environmental cause, intrinsic mutational processes)

UCSF500- TMB in pediatric cancer

688 evaluable cases (T/N)



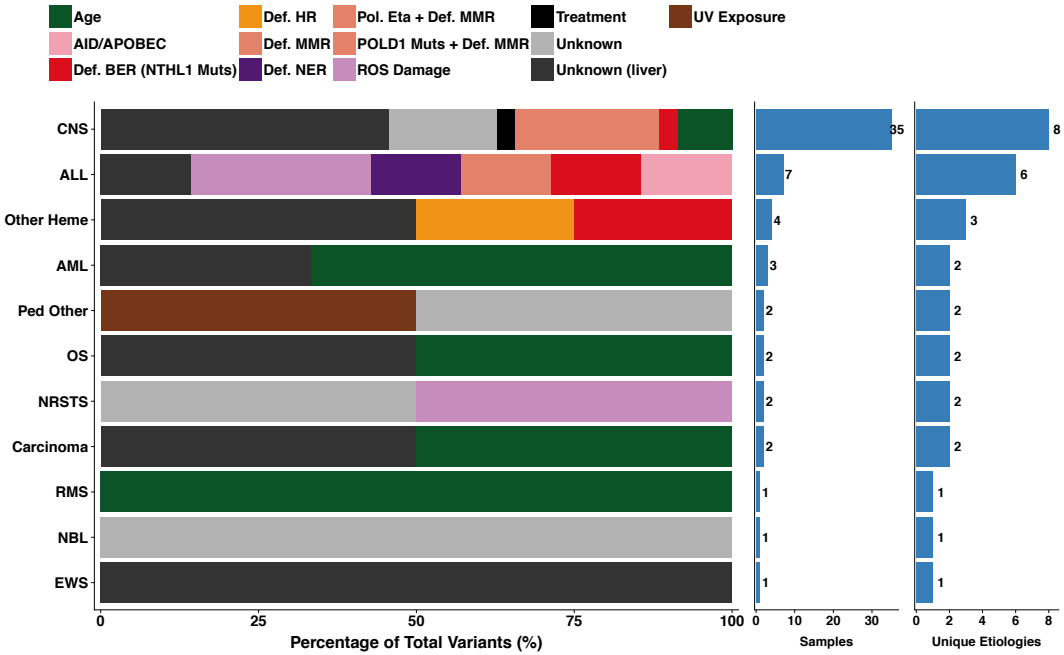
Mutational signatures in cancer



Ma et al Nature Communications 2018

UCSF500- Mutational signatures

Mutational signatures: patterns of single base pair mutations in trinucleotide context provide clues to etiology and tumor evolution



Can only calculate for hyper/ultrahyper TMB (n=60) , otherwise not enough mutations given size of DNA panel.

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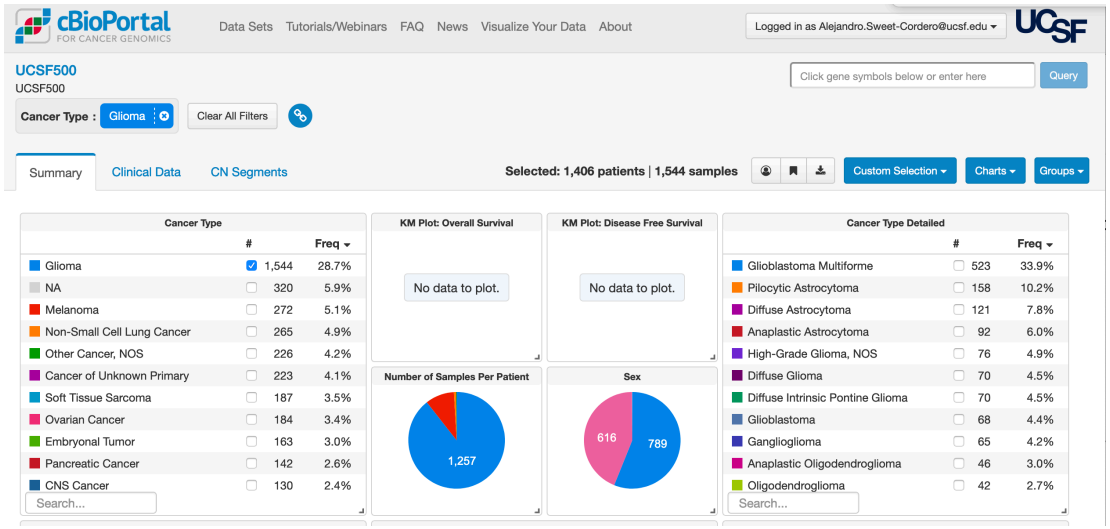
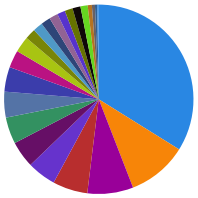
UCSF-Cbioportal





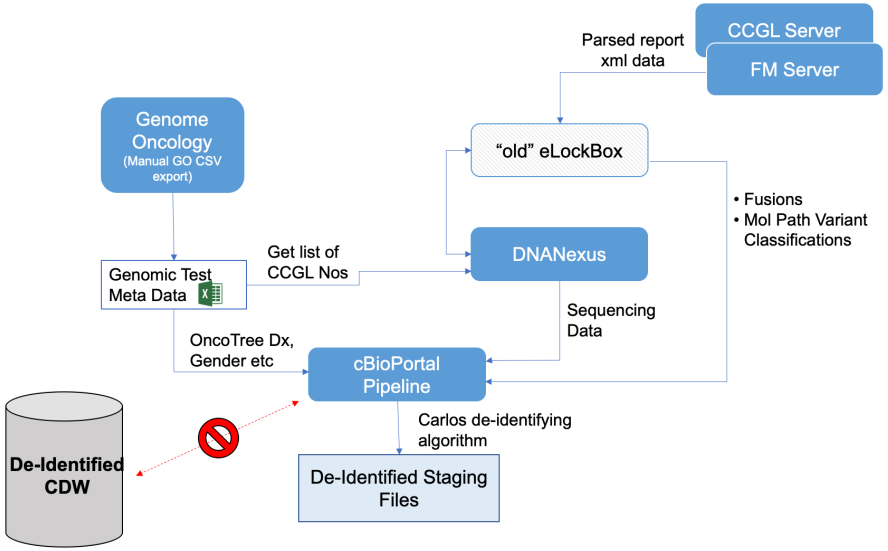
Cancer Type Detailed

- Glioblastoma Multiforme: 523 (33.9%)
- Pilocytic Astrocytoma: 158 (10.2%)
- Diffuse Astrocytoma: 121 (7.8%)
- Anaplastic Astrocytoma: 92 (6.0%)
- High-Grade Glioma, NOS: 76 (4.9%)
- Diffuse Glioma: 70 (4.5%)
- Diffuse Intrinsic Pontine Glioma: 70 (4.5%)
- Glioblastoma: 68 (4.4%)
- Ganglioglioma: 65 (4.2%)
- Anaplastic Oligodendroglioma: 46 (3.0%)
- Oligodendroglioma: 42 (2.7%)
- Encapsulated Glioma: 28 (1.8%)
- Low-Grade Glioma, NOS: 26 (1.7%)
- Anaplastic Pleomorphic Xanthoastrocytoma: 25 (1.6%)
- Gliosarcoma: 23 (1.5%)
- Dysembryoplastic Neuroepithelial Tumor: 21 (1.4%)
- Ependymoma: 21 (1.4%)
- Glioma, NOS: 21 (1.4%)
- Pleomorphic Xanthoastrocytoma: 19 (1.2%)
- Astrocytoma: 11 (0.7%)
- Anaplastic Ganglioglioma: 8 (0.5%)
- Pilomyxoid Astrocytoma: 6 (0.4%)
- Gangliocytoma: 2 (0.1%)
- Oligoastrocytoma: 2 (0.1%)

-Available to all UCSF researchers
-updated quarterly
-wide functionality for data exploration

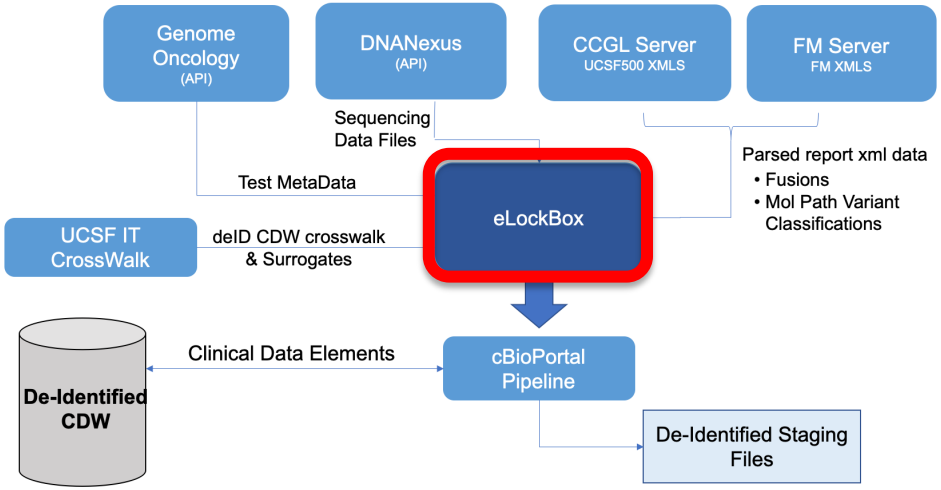
UCSF cBioportal & eLockBox: linking genomics to clinical phenotypes

OLD



- Included manual components
- Relied on pulling, parsing and merging data from multiple sources
- Uncertified de-identifying algorithm
- No linkage to de-identified CDW

NEW



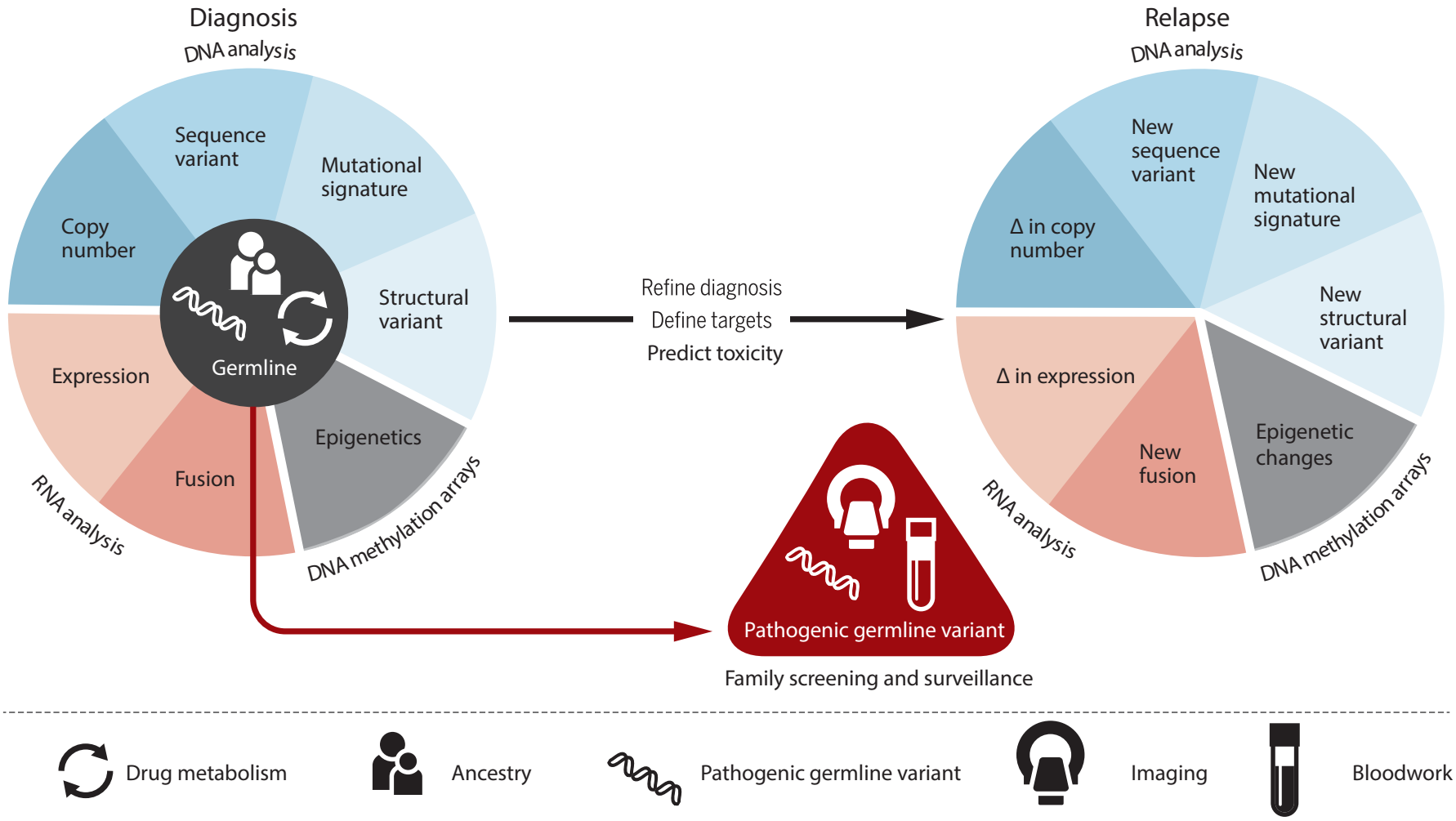
- No manual components
- Exclusively uses eLockBox as source
- Certified surrogates generated by IT using de-identified CDW algorithm
- Can query de-identified CDW directly and pull into cBioPortal any available de-identified clinical data from this source
- Allows us to deploy new data builds with more frequency
  - Every 2-3 months → Every 2-3 weeks (or sooner)

DNA panels in pediatric cancer

- Can clarify the diagnosis (especially with fusion detection)
- Can identify germline predispositions
- Rarely identify actionable SNVs
- Where is the “missing signal” in pediatric cancers?
  - Structural variants in enhancers?
  - Non-coding mutations?
  - Epigenetic alterations?
  - Rare fusions?

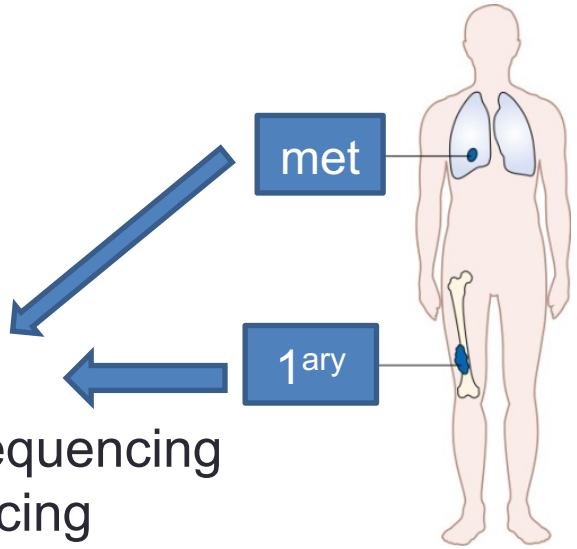
Beyond DNA panels: Integrative WGS/RNAseq

A vision for pediatric cancer genomics

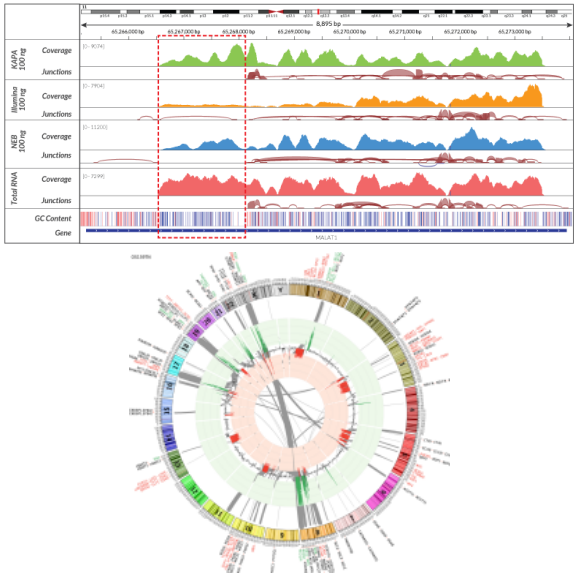


Sweet-Cordero and Biegel, Science 201

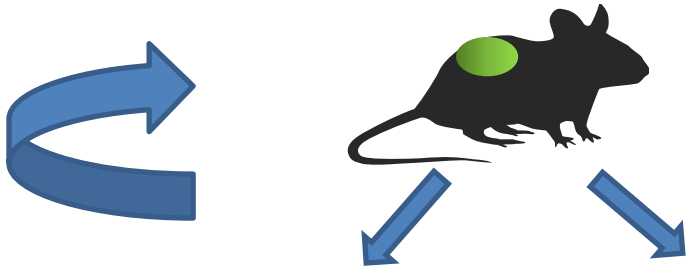
Using patient samples and patient derived xenografts to study pediatric cancers



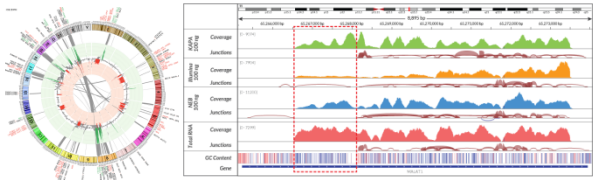
Whole Genome Sequencing
RNA Sequencing



Implant to develop patient-derived xenograft (PDX)

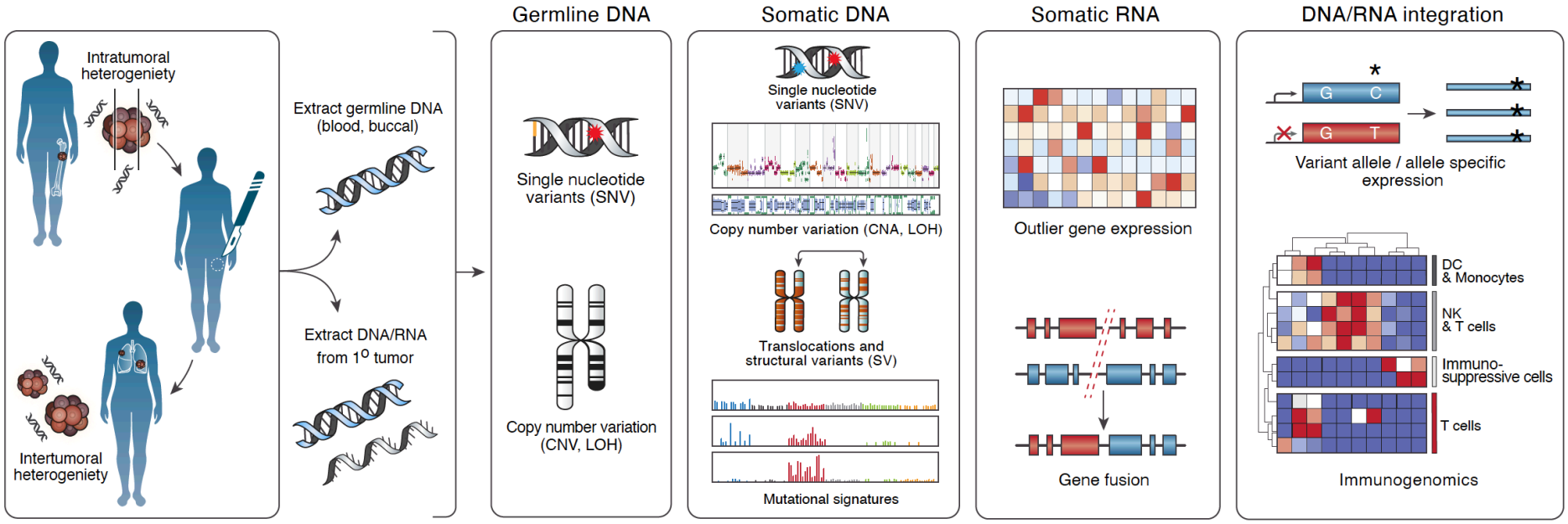


Whole Genome Sequencing
RNA Sequencing

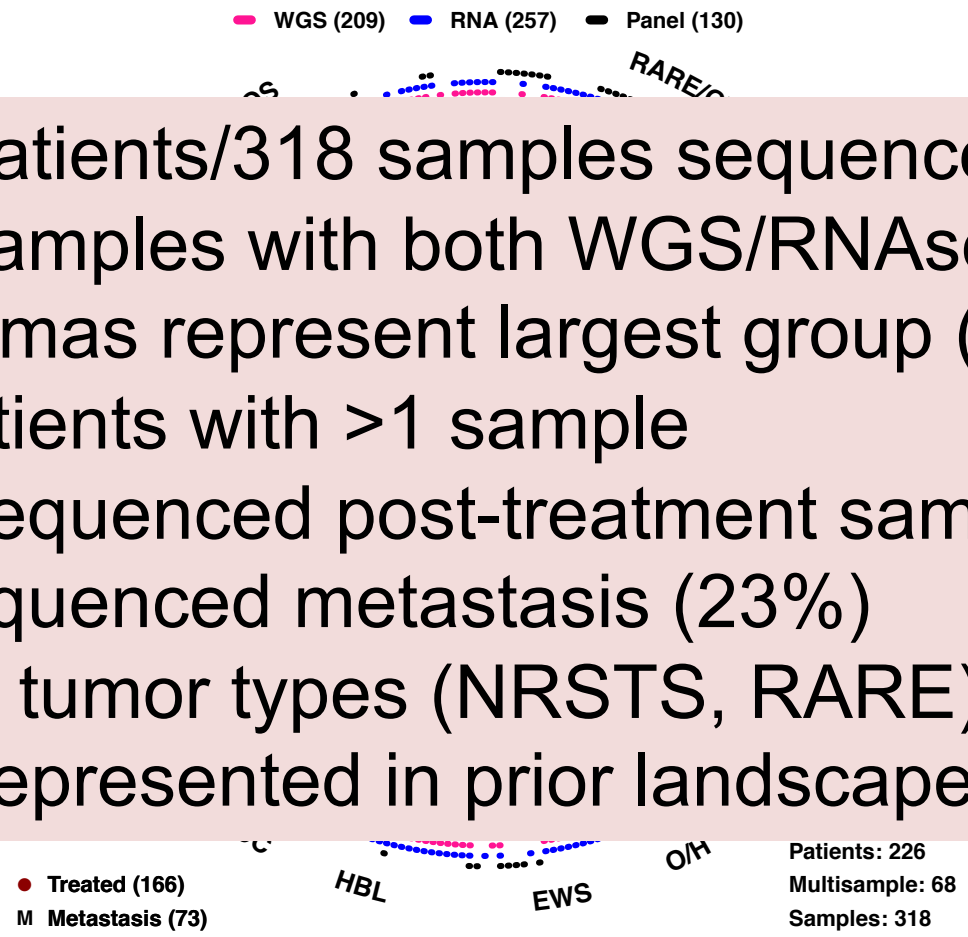


Cell Line Production

Integrative genomics workflow

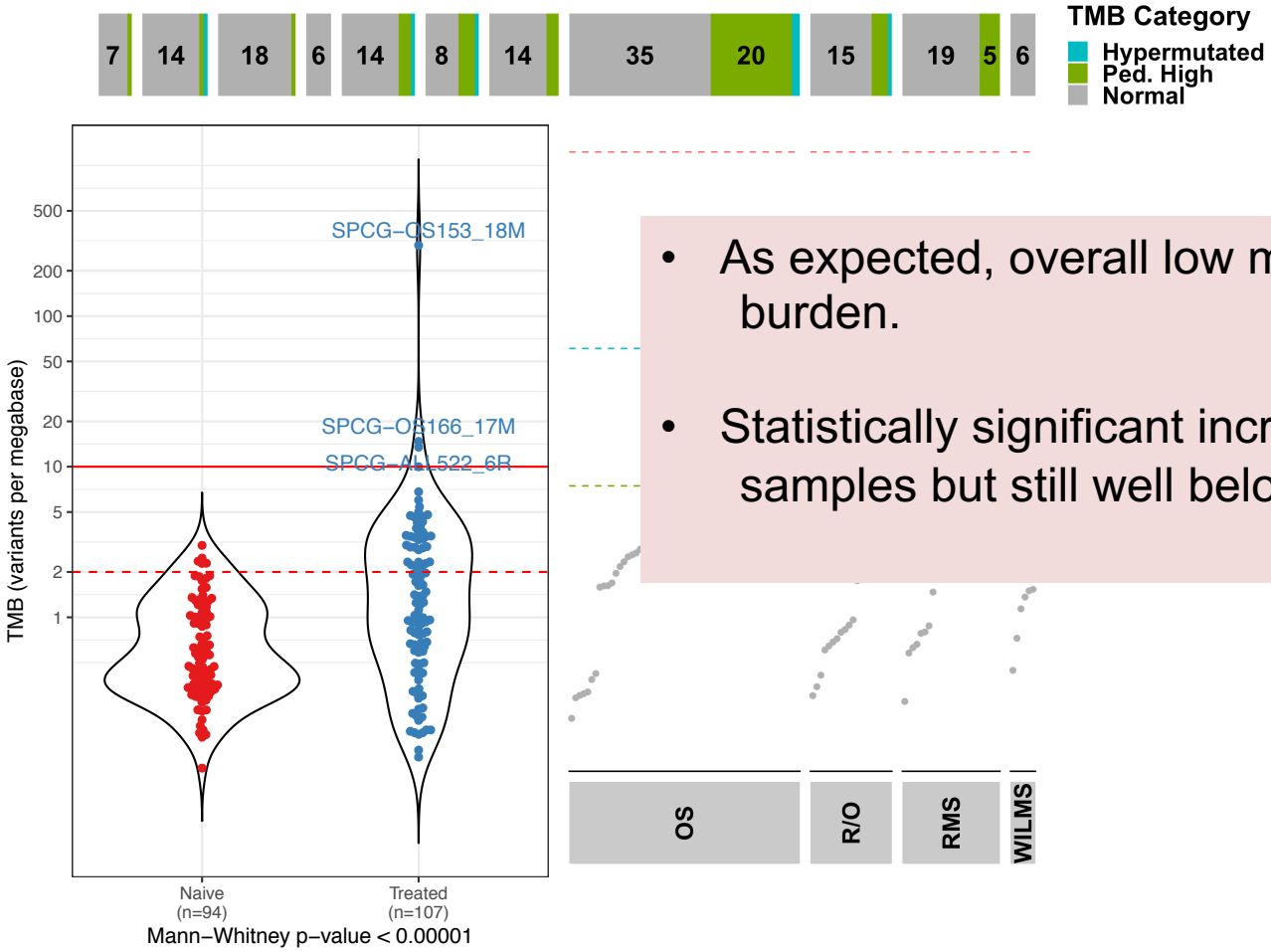


UCSF integrative pediatric cancer sequencing program



- 226 patients/318 samples sequenced
- 175 samples with both WGS/RNAseq
- Sarcomas represent largest group (118 patients)
- 58 patients with >1 sample
- 166 sequenced post-treatment samples (52%)
- 73 sequenced metastasis (23%)
- Some tumor types (NRSTS, RARE) not well-represented in prior landscape efforts

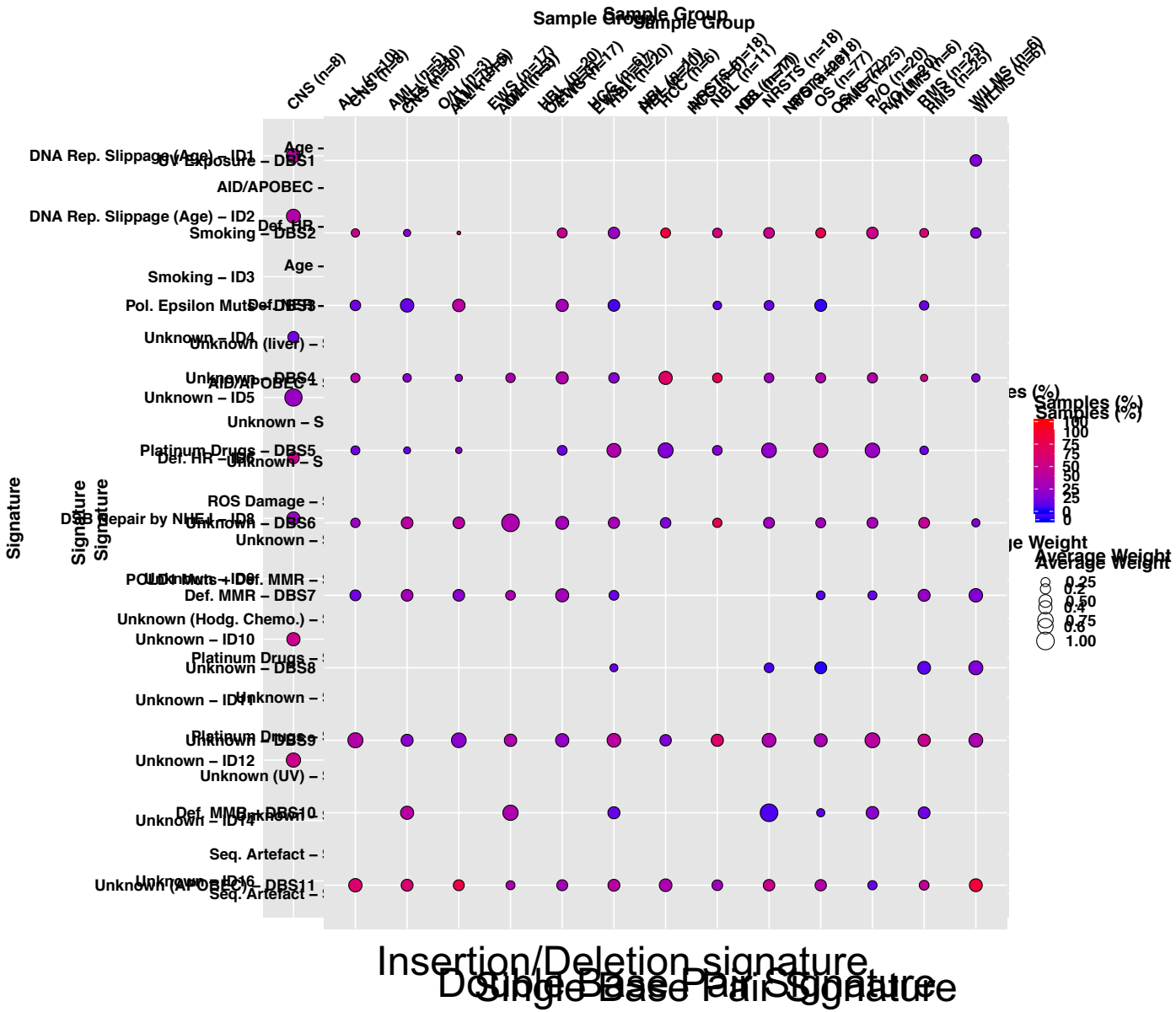
Mutational burden in pediatric cancer



- As expected, overall low mutational burden.
- Statistically significant increase in treated samples but still well below most adult cancers

- Only 3% ultra/hypermutated
- Larger fraction “pediatric high” but clinial relevance unclear

Mutational signatures using WGS

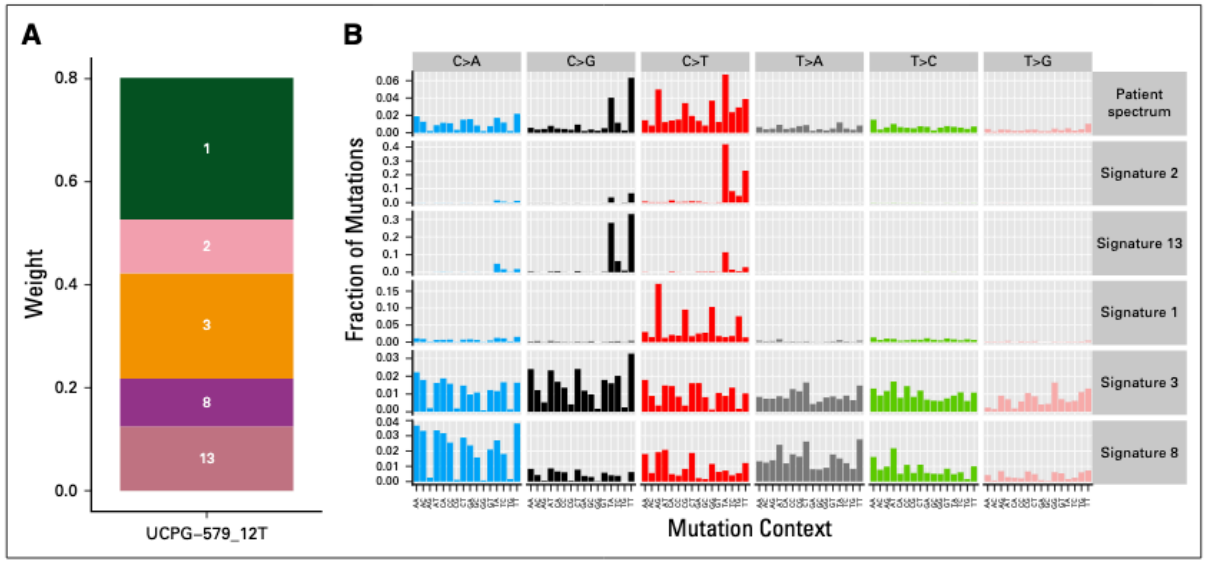


Retrospective analysis of an exceptional responder

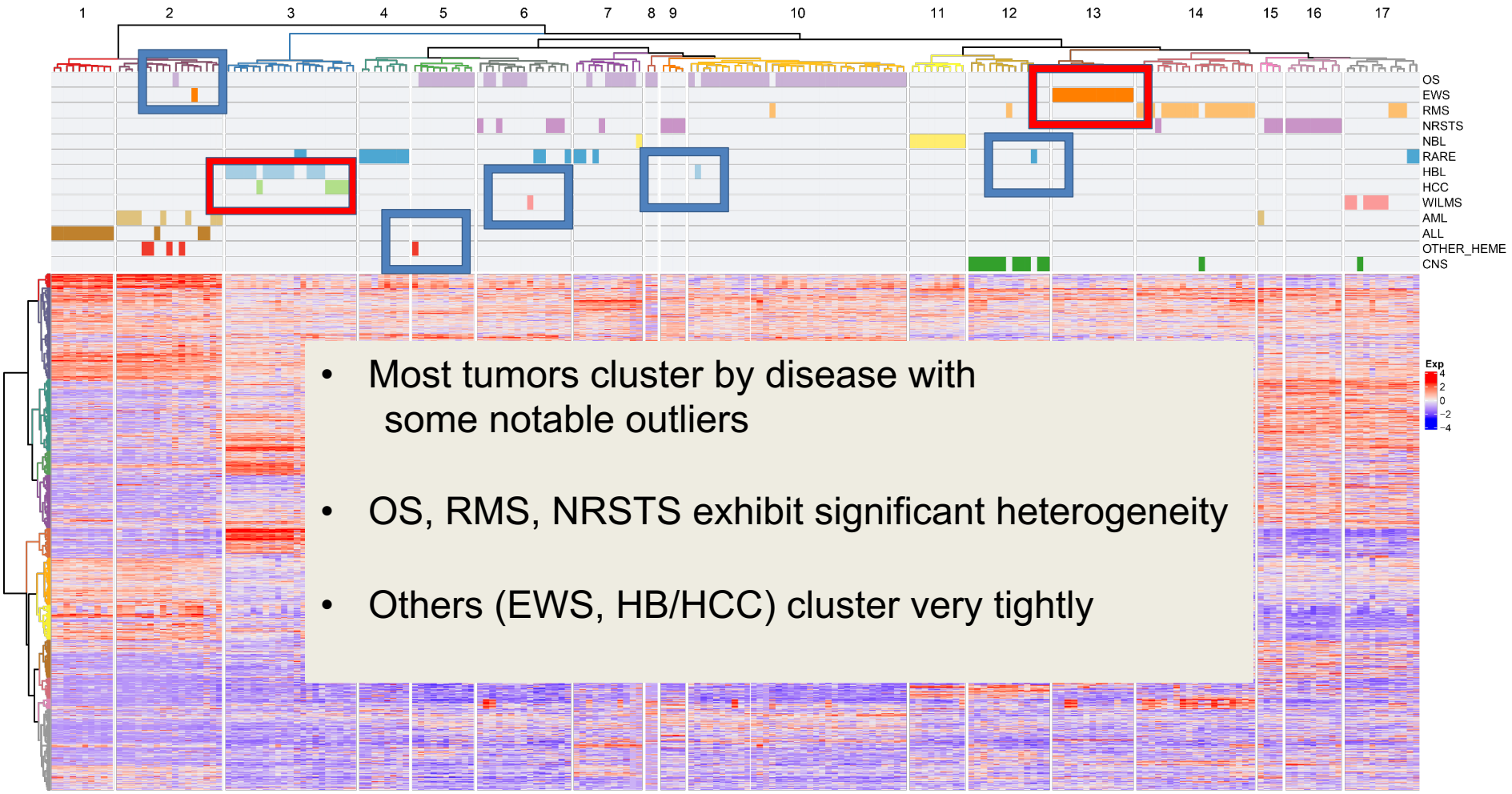
case reports

Complete Response to PD-1 Inhibition in an Adolescent With Relapsed Clear Cell Adenocarcinoma of the Cervix Predicted by Neoepitope Burden and APOBEC Signature

Anya Levinson, MD[1]; Alex G. Lee, PhD[1]; Henry J. Martell, PhD[1]; Marcus R. Breese, PhD[1]; Charles Zaloudek, MD[2,3]; Jessica Van Ziffle, PhD[3]; Benjamin Laguna, MD[4]; Stanley G. Leung, BA[1]; M. Dwight Chen, MD[5]; Lee-may Chen, MD[2,6]; Jacob Pfeil, PhD[7,8]; Nicholas R. Ladwig, MD[3]; Avanthi Tayi Shah, MD[1]; Inge Behroozfard, BS[1]; Arjun Arkal Rao, PhD[3]; Sofie R. Salama, PhD[7,9]; E. Alejandro Sweet-Cordero, MD[1,2]; and Elliot Stieglitz, MD[1,2]



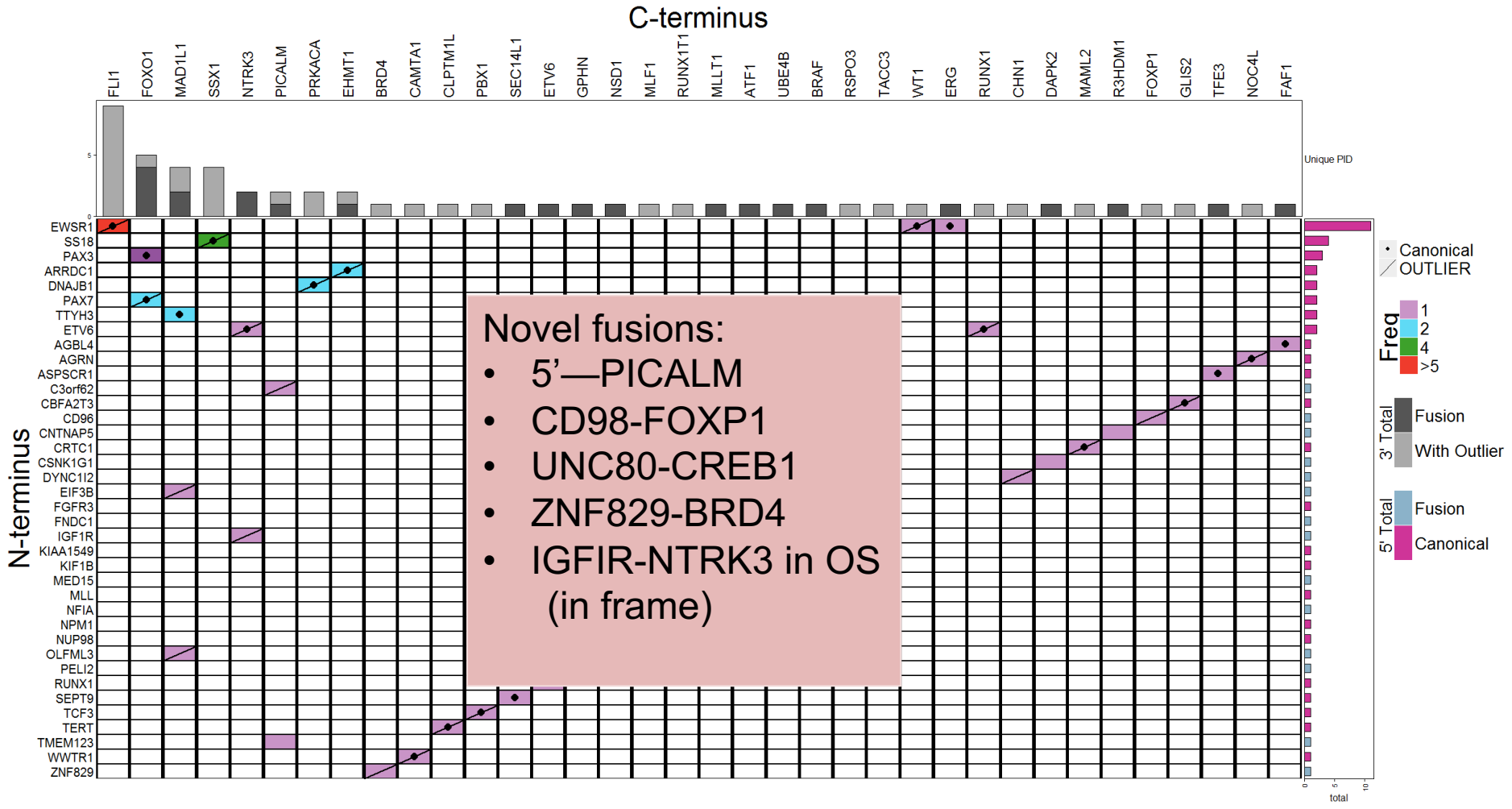
The transcriptome of pediatric cancers



- Most tumors cluster by disease with some notable outliers
- OS, RMS, NRSTS exhibit significant heterogeneity
- Others (EWS, HB/HCC) cluster very tightly

Clustering of 4343 most variable genes in 235 clinical samples (75% top variable genes)

RNAseq identifies known and novel fusion genes

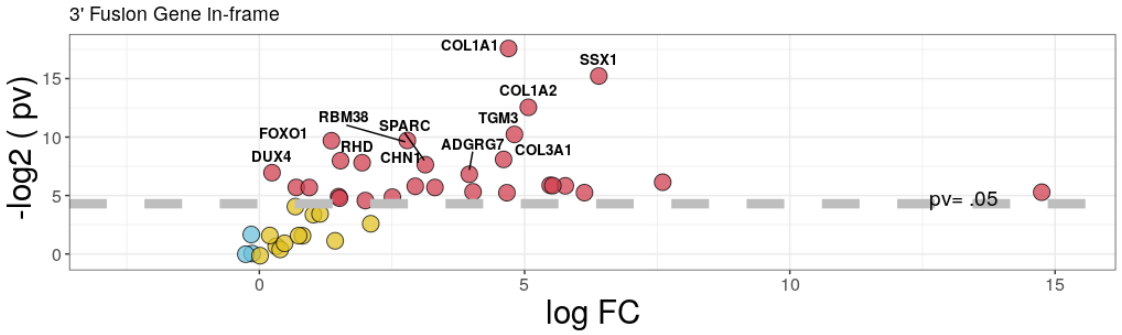


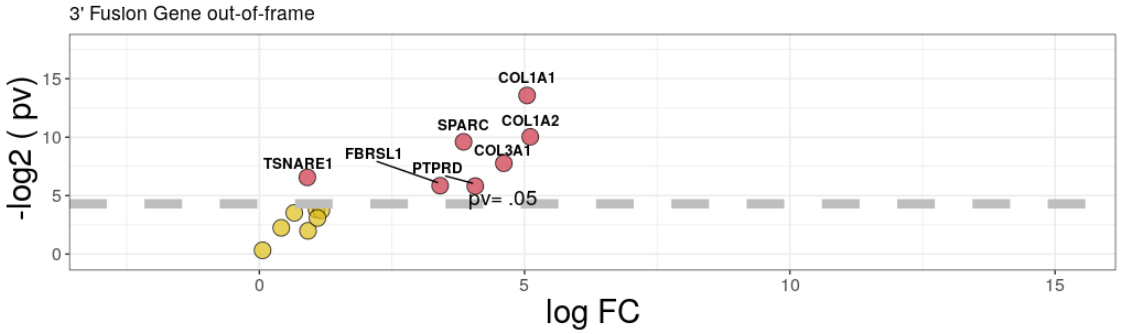
Novel fusions:

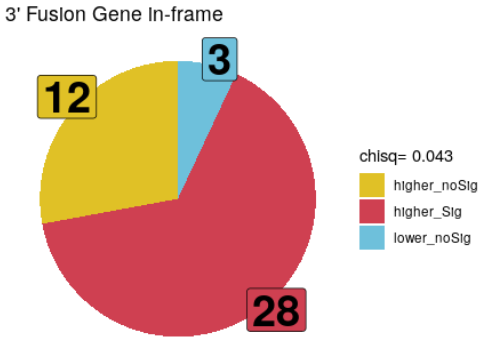
- 5'—PICALM
- CD98-FOXP1
- UNC80-CREB1
- ZNF829-BRD4
- IGFIR-NTRK3 in OS (in frame)

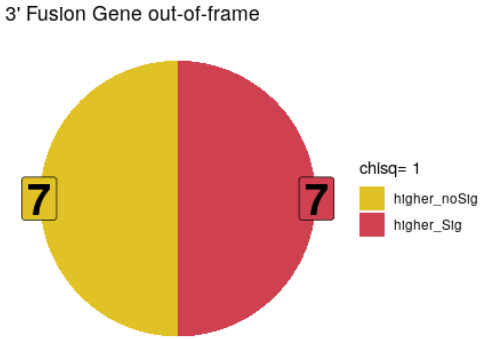
Outlier gene expression suggests that many rare fusions may be drivers genes

Outlier gene expression nominates novel oncogenic fusions









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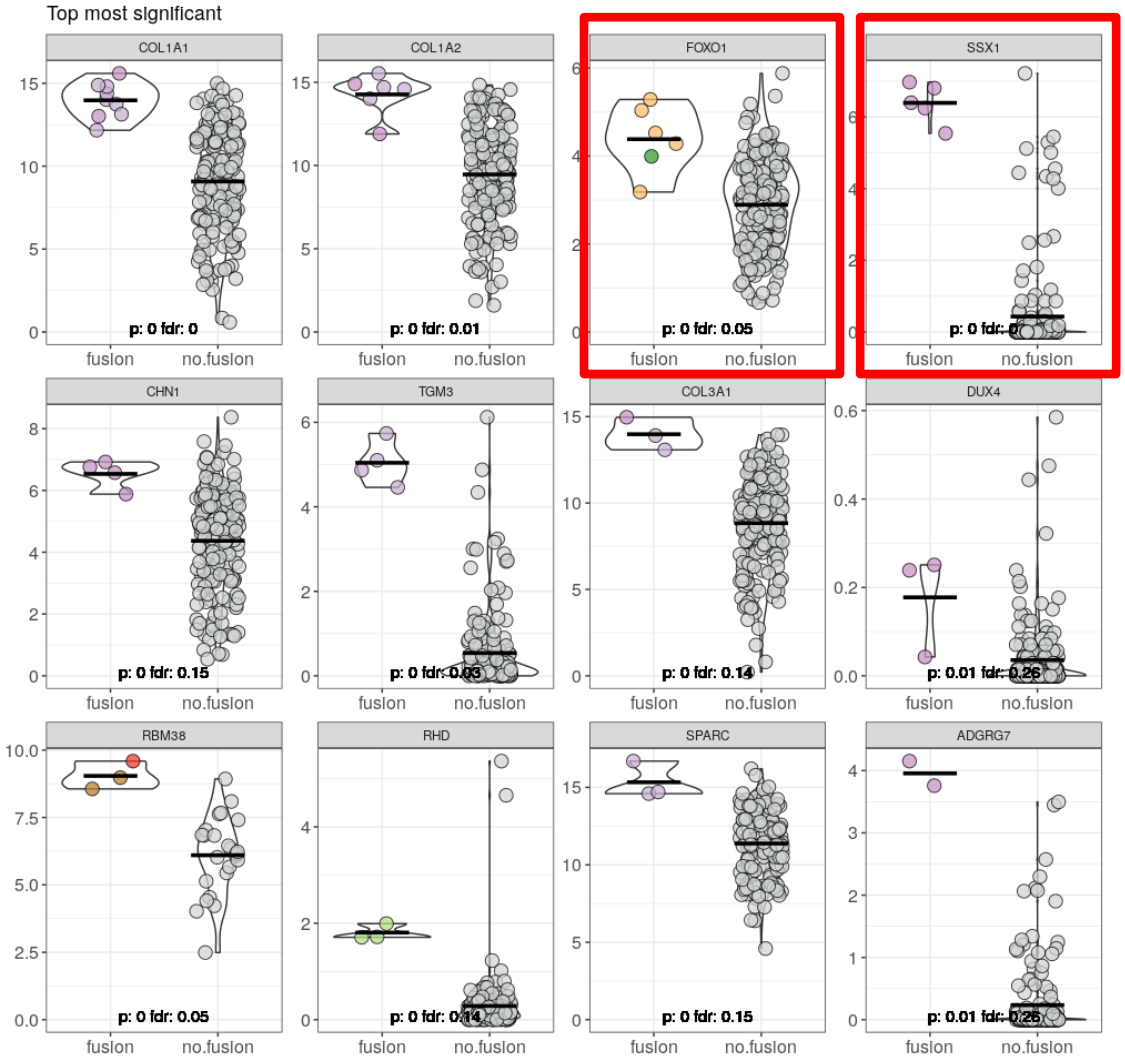
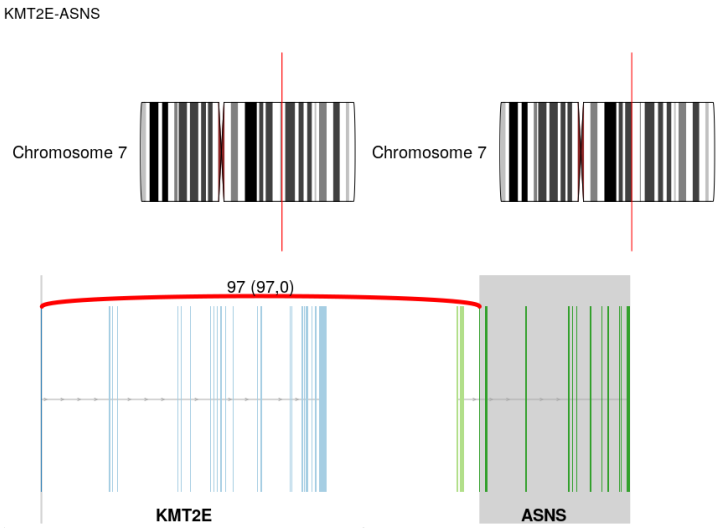
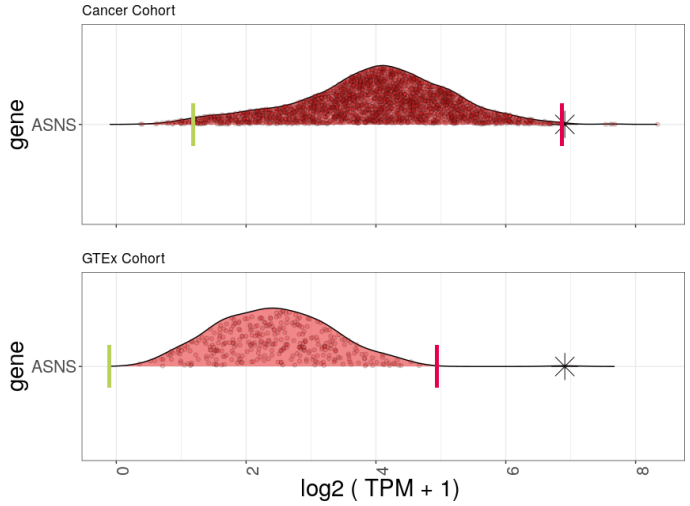


Known

ASNS fusion as a mechanism for asparaginase resistance



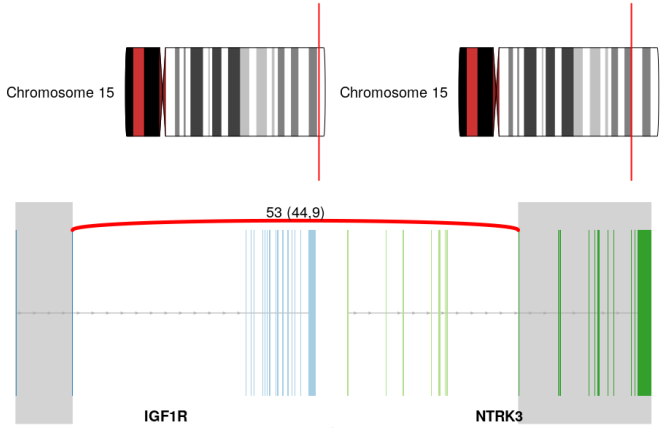
Fusion detected by RNAseq



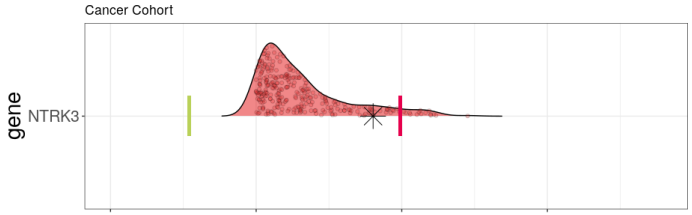
Elevated ASNS expression

- Relapsed leukemia sample, prior treatment with asparagine
- Novel mechanism of resistance involving fusion upregulating asparagine synthase (ASNS) so cells are now insensitive to asparagine depletion

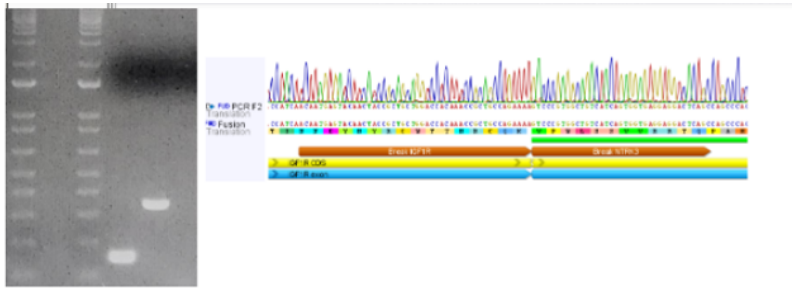
NTRK3 fusion in a patient with osteosarcoma



Fusion detected by RNAseq

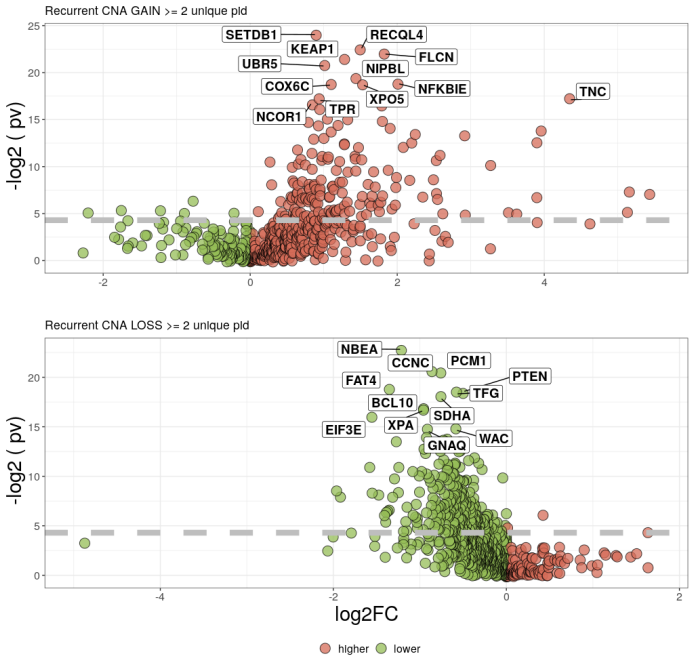


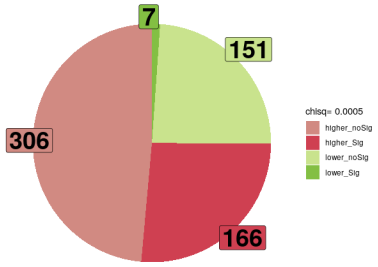
Elevated NTRK3 expression

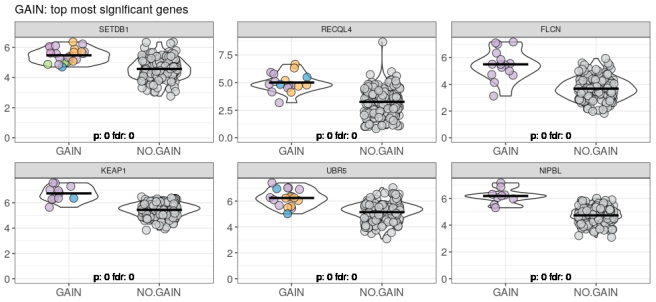


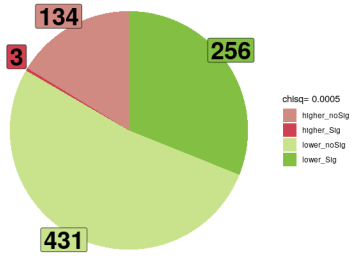
NTRK3 fusion confirmed by PCR/Sanger sequencing

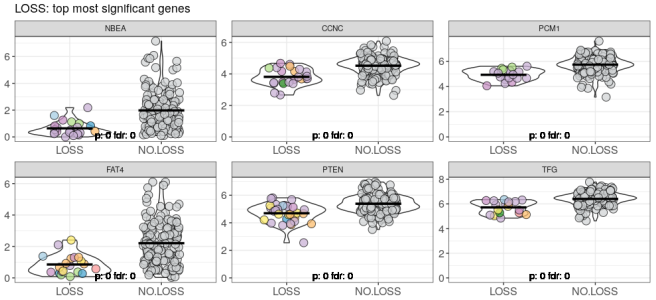
Integration of expression with Copy number change



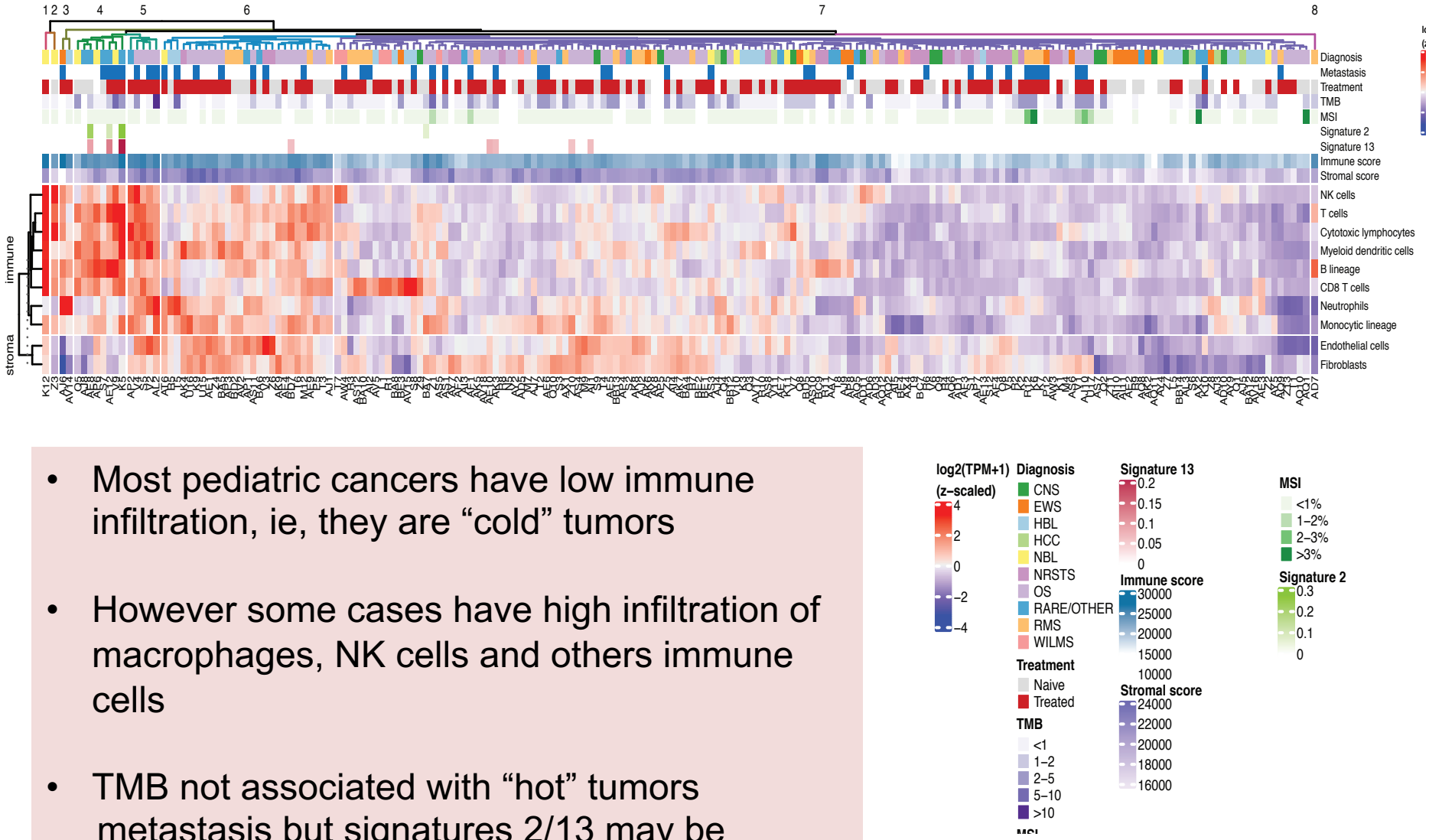






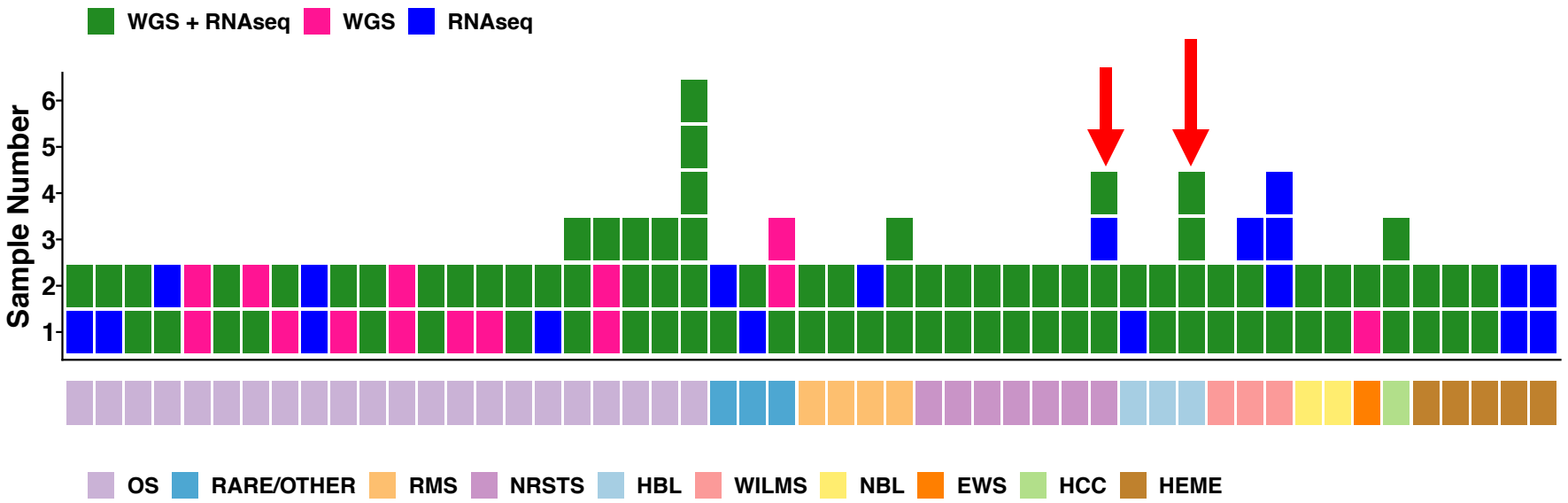


In silico immunoprofiling of pediatric solid tumors



- Most pediatric cancers have low immune infiltration, ie, they are “cold” tumors
- However some cases have high infiltration of macrophages, NK cells and others immune cells
- TMB not associated with “hot” tumors metastasis but signatures 2/13 may be associated?

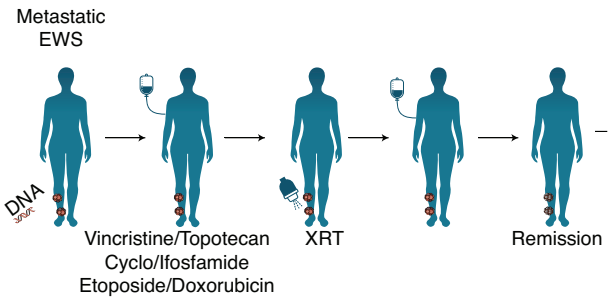
Longitudinal cohort



- How do pediatric cancers progress from diagnosis through metastasis?
- Are there recurrent alterations present? Are these actionable?

Case 1: Ewing followed by radiation-induced sarcoma

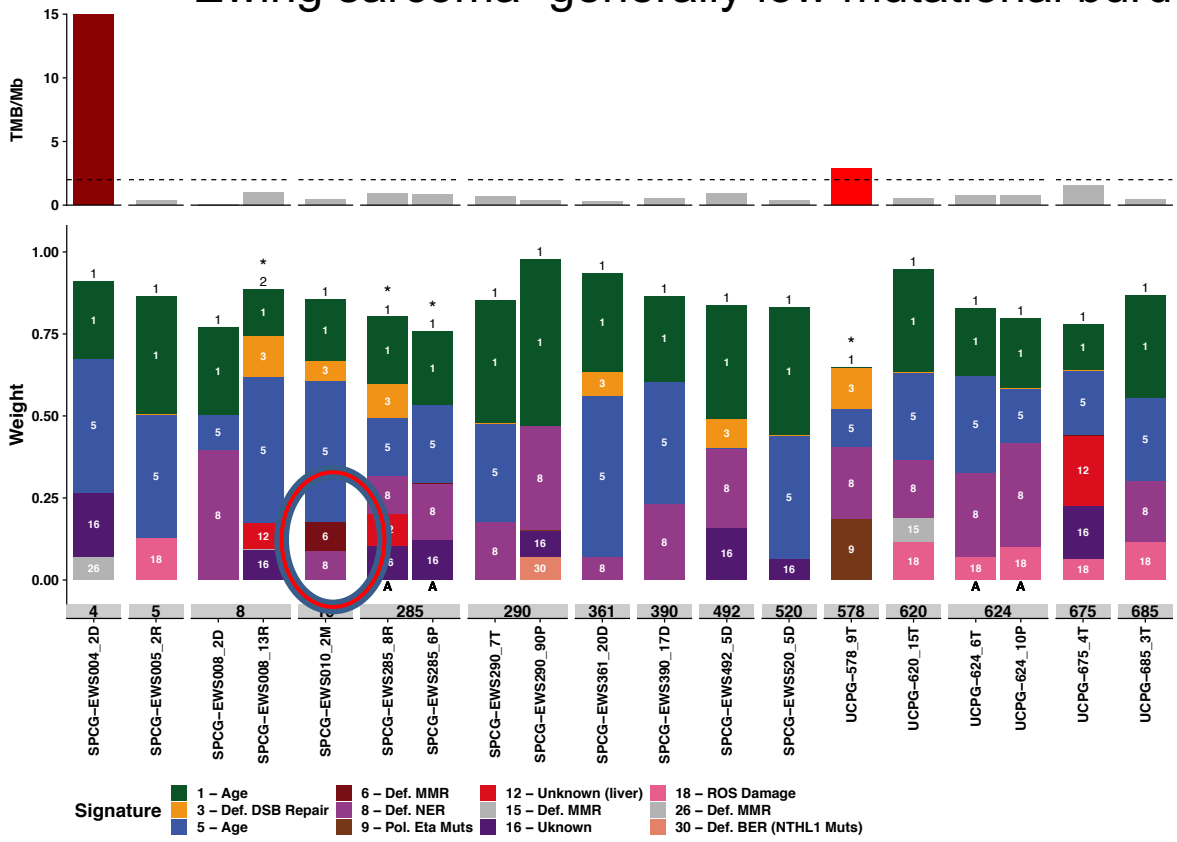
- Diagnosed age 9->metastatic Ewing Sarcoma.
- Received systemic chemotherapy and radiation to local site.
- Relapsed 5 years later with sarcoma at radiation site
- Metastasis to lung
- More chemotherapy
- Deceased



Longitudinal genomic analysis: what are the consequences of repeated cycles of DNA da

Ewing sarcoma mutational signature at diagnosis

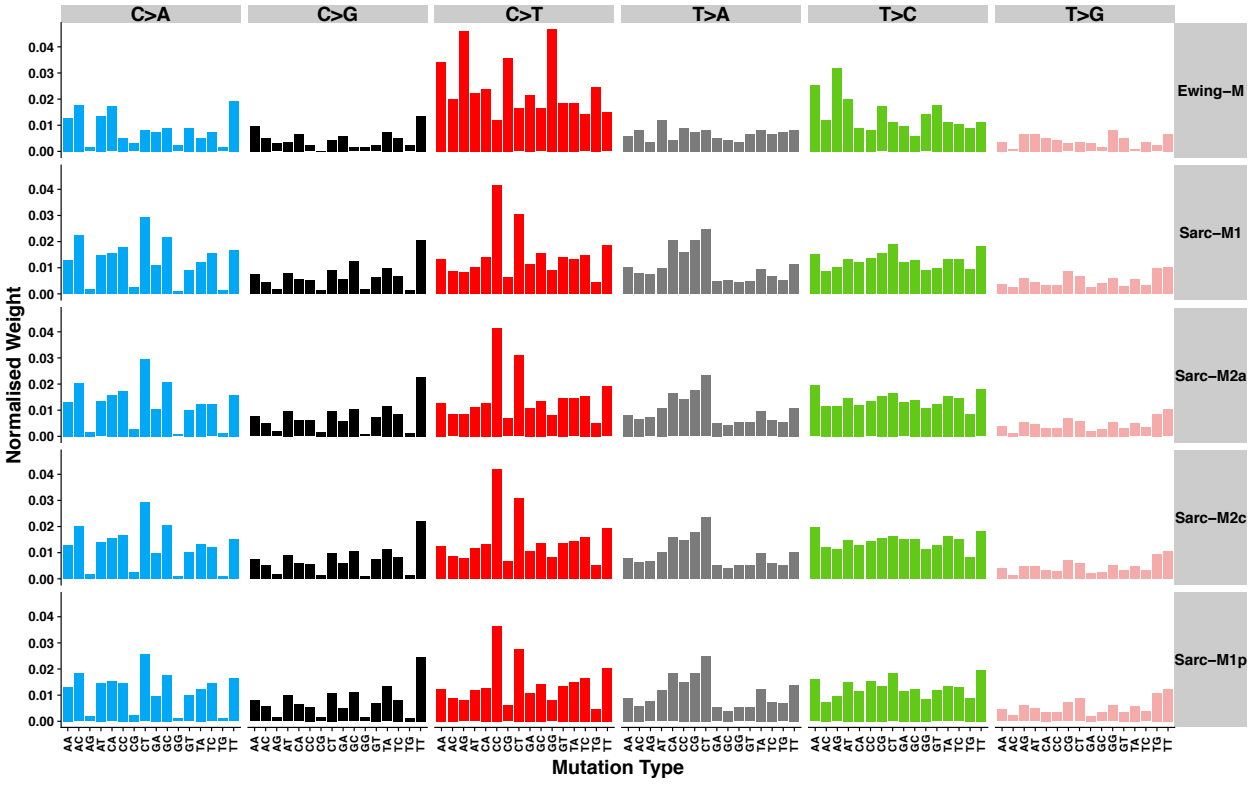
Ewing sarcoma=generally low mutational burden



Unusual presence of MMR signature might have suggested increased response to immune checkpoint blockade (not known at the time)

Henry Martell

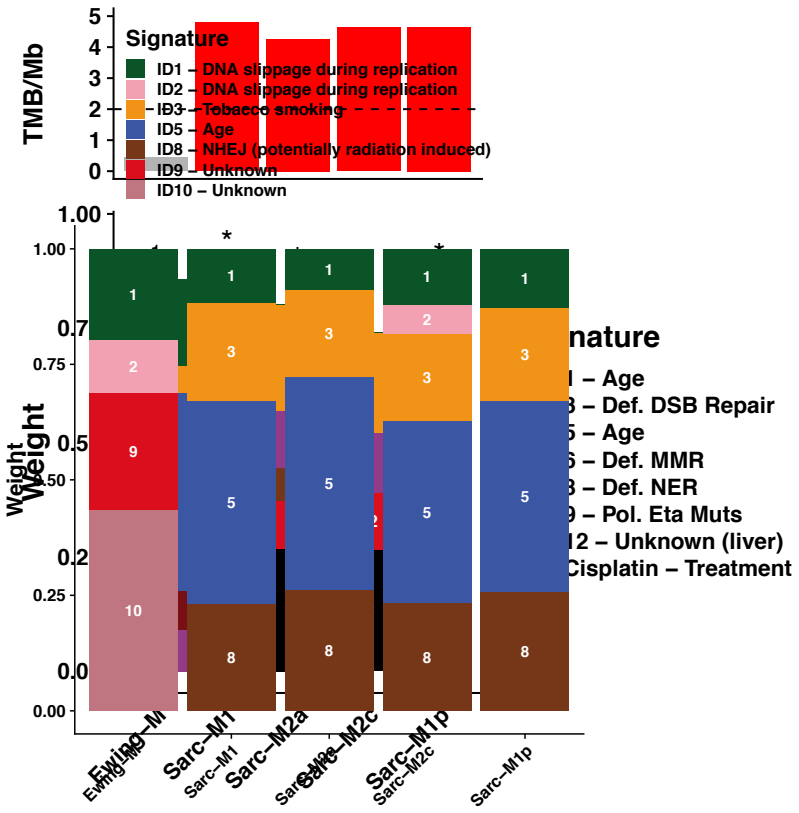
Mutational signatures acquired during DNA damaging therapy in a single patient



(normalized)

Henry Martell

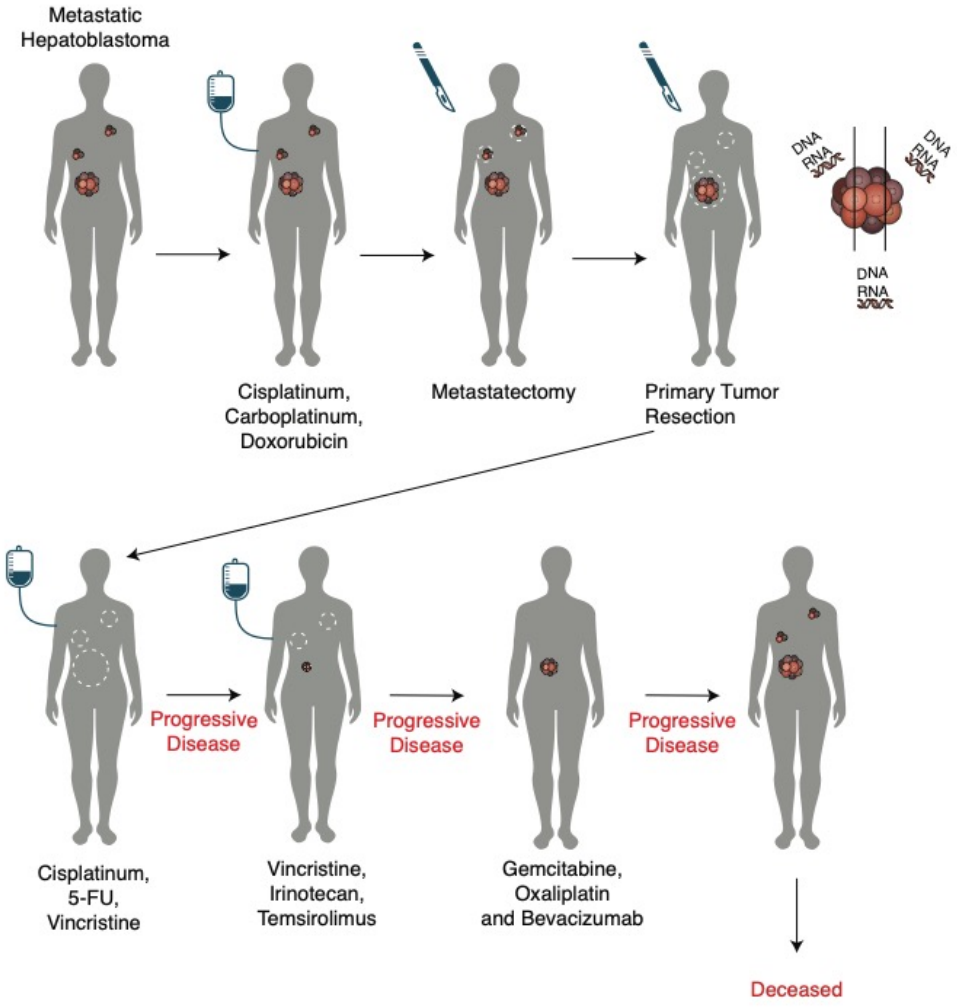
Mutational signatures acquired during DNA damaging therapy in a single patient



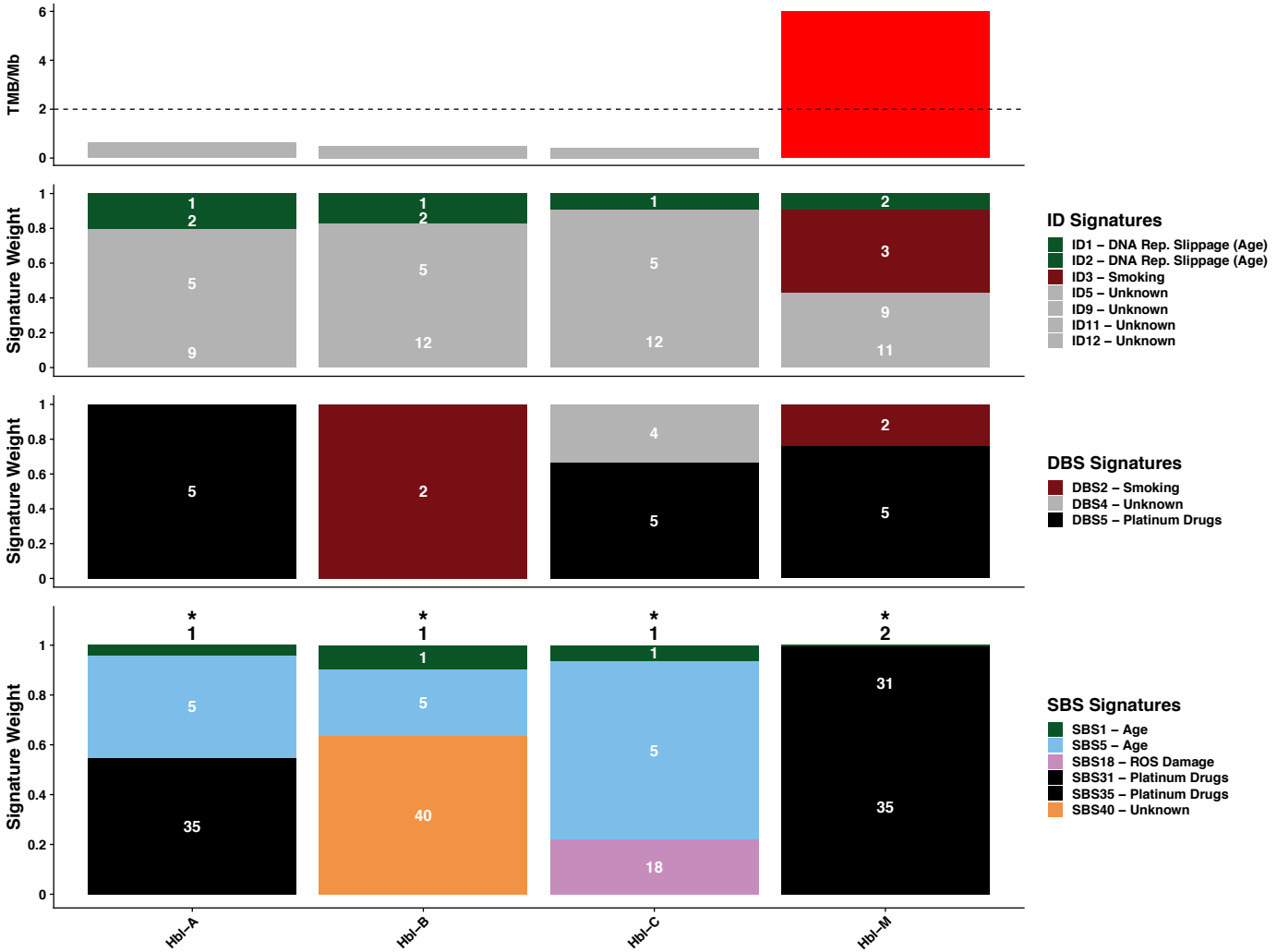
- Increased tumor mutational burden at relapse
- Emergence of a cisplatin mutational signature.
- Emergence of a PolE signature. Checkpoint response?
- Emergence of a NHEJ signature and new indel signatures, likely reflecting DSB repair after radiation.
- Highly informative WGS…yet this is still not standard of care even for complex cases like this one..

Henry Martell

Case 2: Metastatic hepatoblastoma

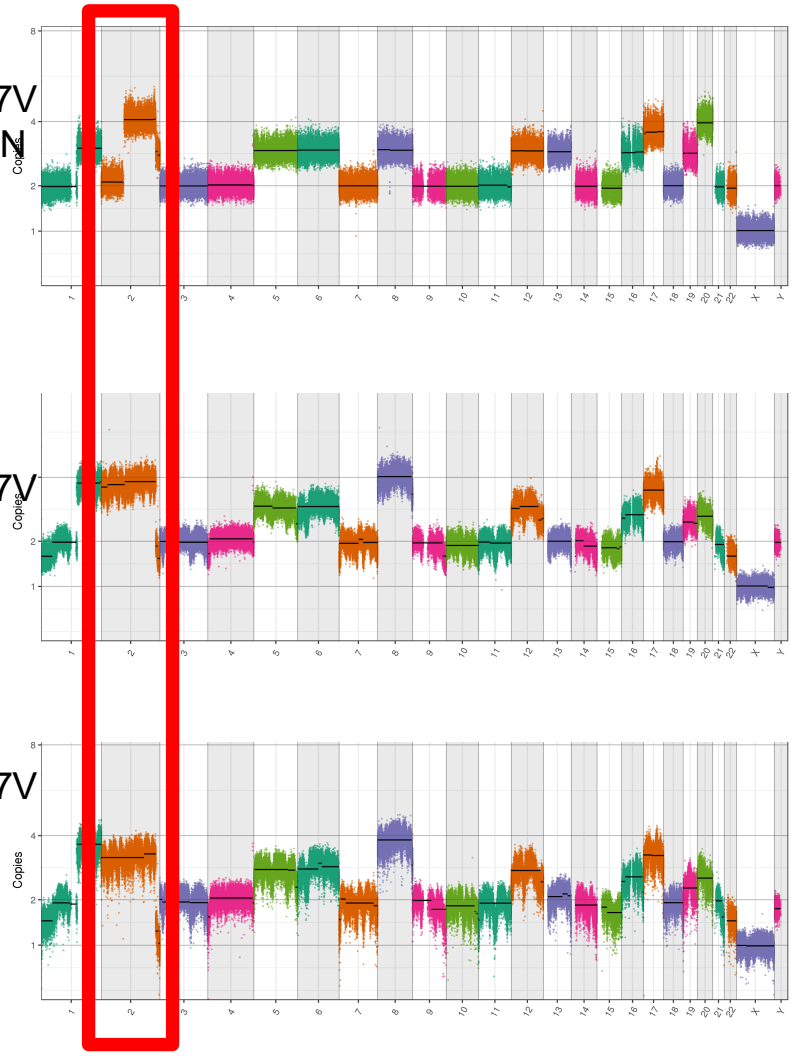


Case 2: evolution of mutational signatures



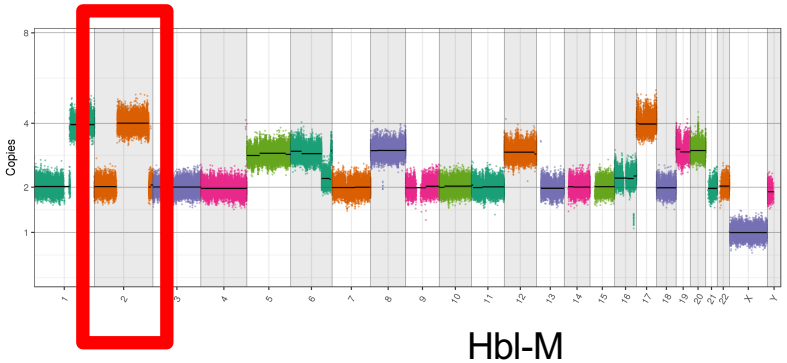
Copy number and SNV in Hepatoblastoma progression

Hbl-A
CTNNB1:G27V
GPR142:K11N



Hbl-B
CTNNB1:G27V

Hbl-C
CTNNB1:G27V



Hbl-M
CTNNB1:G27V
GPR142:K11N
CNTNAP2:L15Q

Summary-Part1

- DNA panel assays provide significant support in defining germline alterations, clarifying diagnosis and in some cases identifying druggable alterations in pediatric cancers
- Most pediatric cancers lack clearly actionable alterations suggesting need for additional molecular characterization of tumors
- TMB is generally low in pediatric cancers but some tumors have high TMB and mutational signatures can identify potentially therapeutically relevant signatures
- RNAseq has the potential to increase the identification of actionable and potentially druggable alterations in pediatric cancer patients.
- A significant fraction of pediatric cancers may have rare fusion events that serve as oncogenic drivers or mediators of resistance

Other ASC lab computational efforts.

- Genomic evolution of osteosarcoma (collaboration with Christina Curtis)

  -Integration of WGS with Bionano data

- Analysis of chromosome accessibility changes during metastatic progression using ATAC-seq

  -Integration of ATAC-seq with RNAseq

- scRNAseq analysis of response to targeted therapies in GEM models of lung cancer.

Molecular Oncology Initiative Core TEAM

- **Discussants**:
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  - Beth Apsel Winger, MD, PhD
  - Jessica Schulte, MD
  - Elliot Stieglitz, MD
  - Alejandro Sweet-Cordero, MD

- **Other collaborators:**
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  - Jennifer Grabowski, PharmD (pharmacist)
  - Marta Sabbadini, PhD (genetic counselor)

- **Program Manager:**
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- **Clinical Research Coordinator:**
  - Ana Quintanar Alfaro, BS
- **Genomics leadership:**
  - Aleks Rajkovic, MD, PhD

- **Computational Biologists**:
  - Carlos Espinosa, BA/BS
  - Courtney Onodera, PhD

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San Francisco

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