La Cérémonie d'attrubution du titre de
Docteur Honoris Causa de l'Université d'Évry-Val-d'Essonne

Cancer Genomics: Foundations and Future

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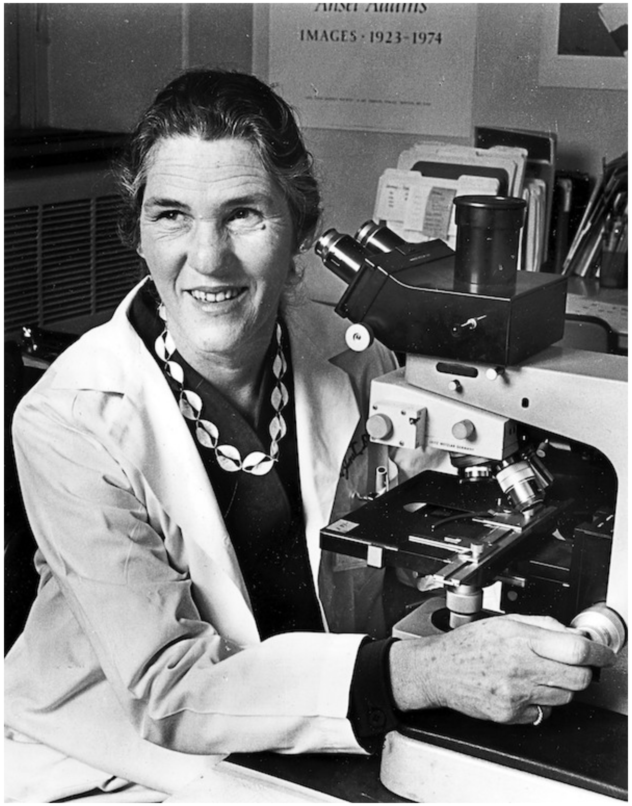
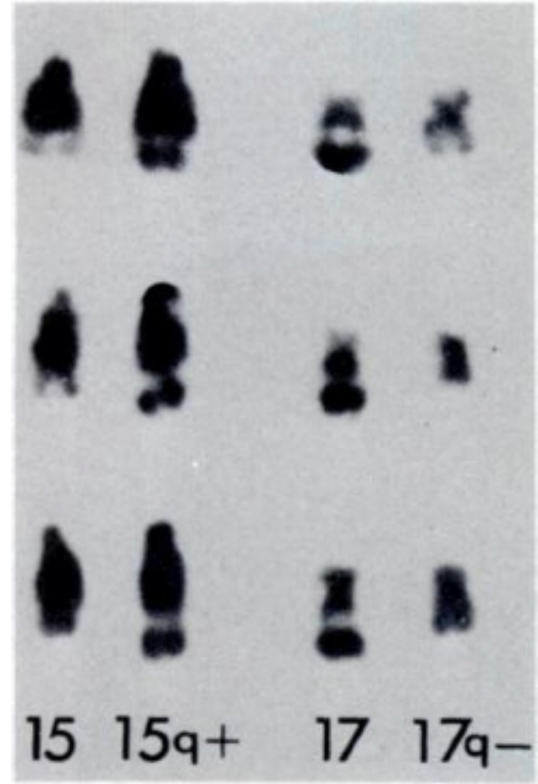
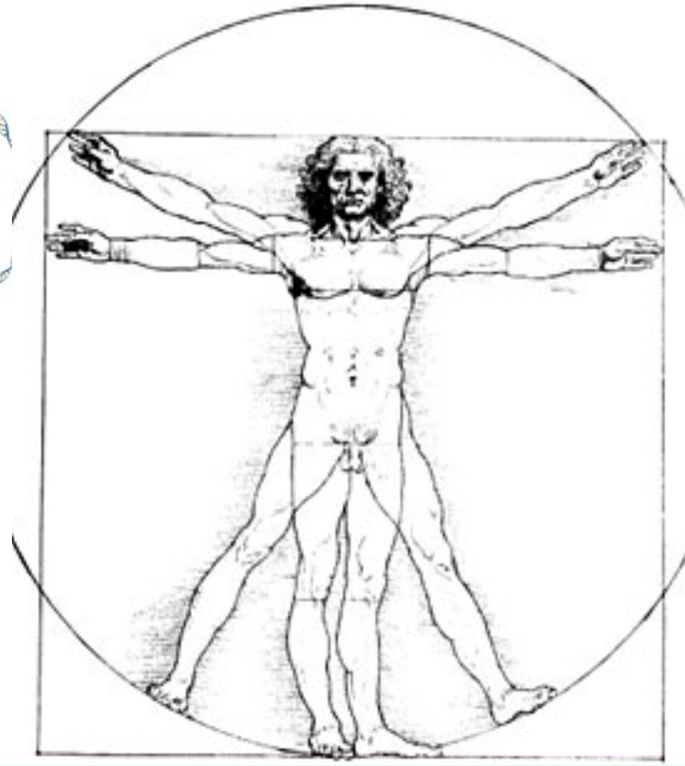
Cancer is a Disease of the Genome





In the early 1970's, Janet Rowley's microscopy studies of leukemia cell chromosomes suggested that specific DNA-based alterations led to cancer, laying the foundation for cancer genomics.

The Human Genome



Human Genome Project
1989-2003

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The Human Genome enables Cancer Genomics

- The human genome reference sequence is the keystone for interpreting NGS sequencing read data
- <u>**Alignment**</u> of reads to the human reference sequence is the first step to identify variation of all types: first we align tumor and normal separately, identify variants in each and then compare the two to derive somatic and germline alterations
- Alignment and analysis of RNA sequence data provides information about gene and isoform/allele expression

Single Nucleotide Variants

Insertion/deletions

Structural Variants

Copy Number Variations

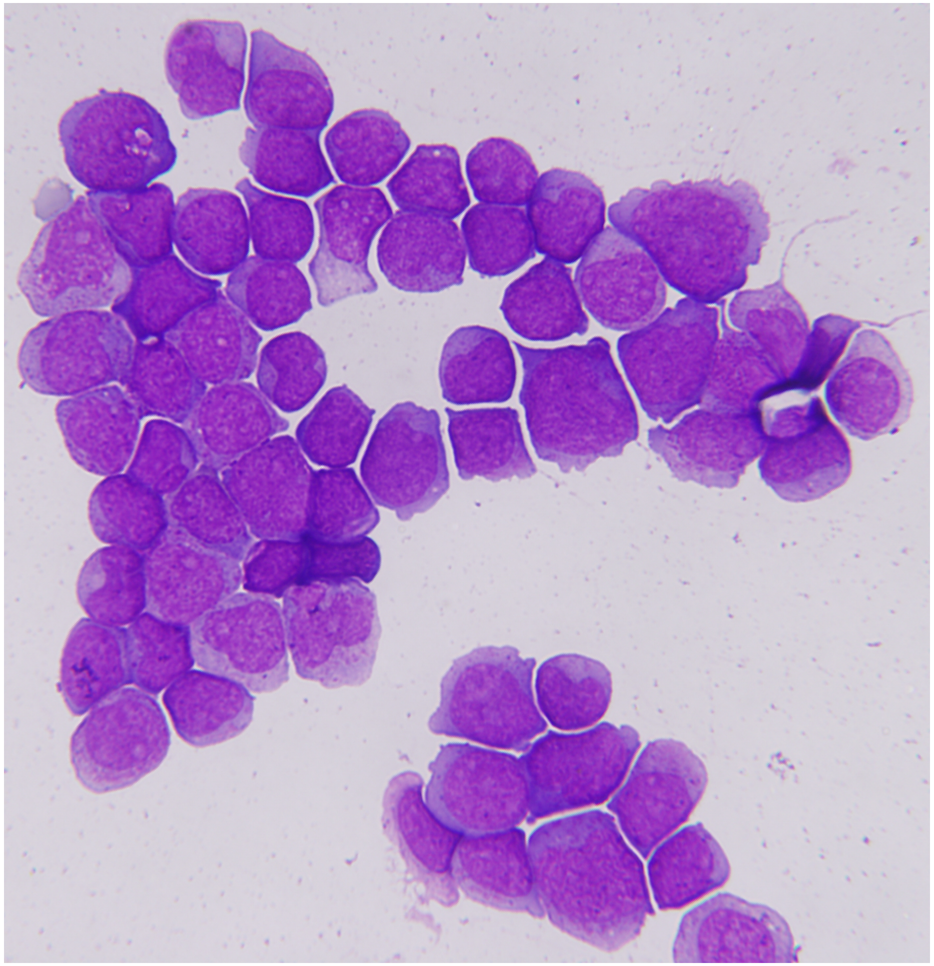
Allele-specific expression

Differentially Expressed Genes

Differentially Expressed Isoforms

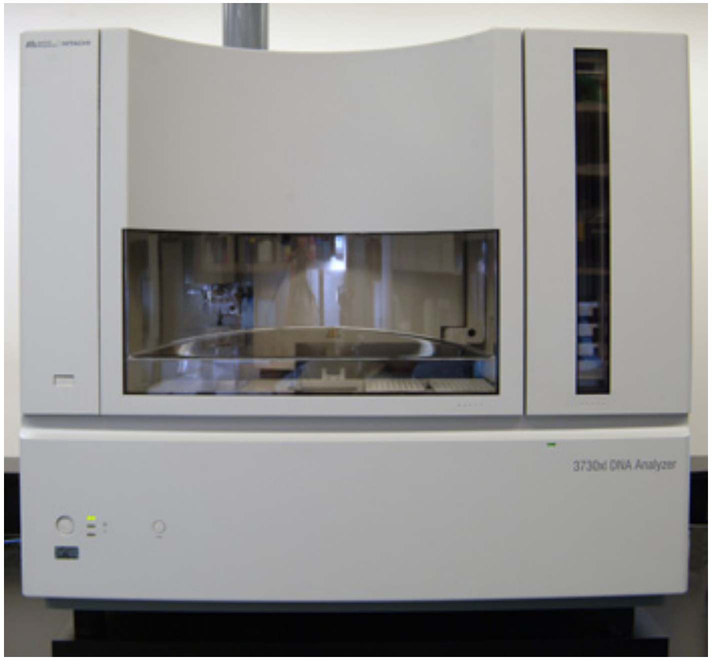
The first NGS cancer whole genome sequence (2008)

- Caucasian female, diagnosis in mid-50s, *de novo* M1 AML
- Normal cytogenetics (chromosomes) and a family history of cancer
- Our analyses identified only 10 genes with mutations (2 known previously in AML)
- Data generation took 9 months and cost around **$1M** but established a paradigm for the use of NGS to identify somatic alterations in an unbiased manner



Ley et al., Nature 2008

Whole human genome sequencing costs



Capillary technology

Applied Biosystems 3730xl (2004)

$15,000,000/genome

~5 years, International project

Next-gen technology

Illumina NovaSeq (2017)

$1,000/genome

~44 hours@20 genomes per run

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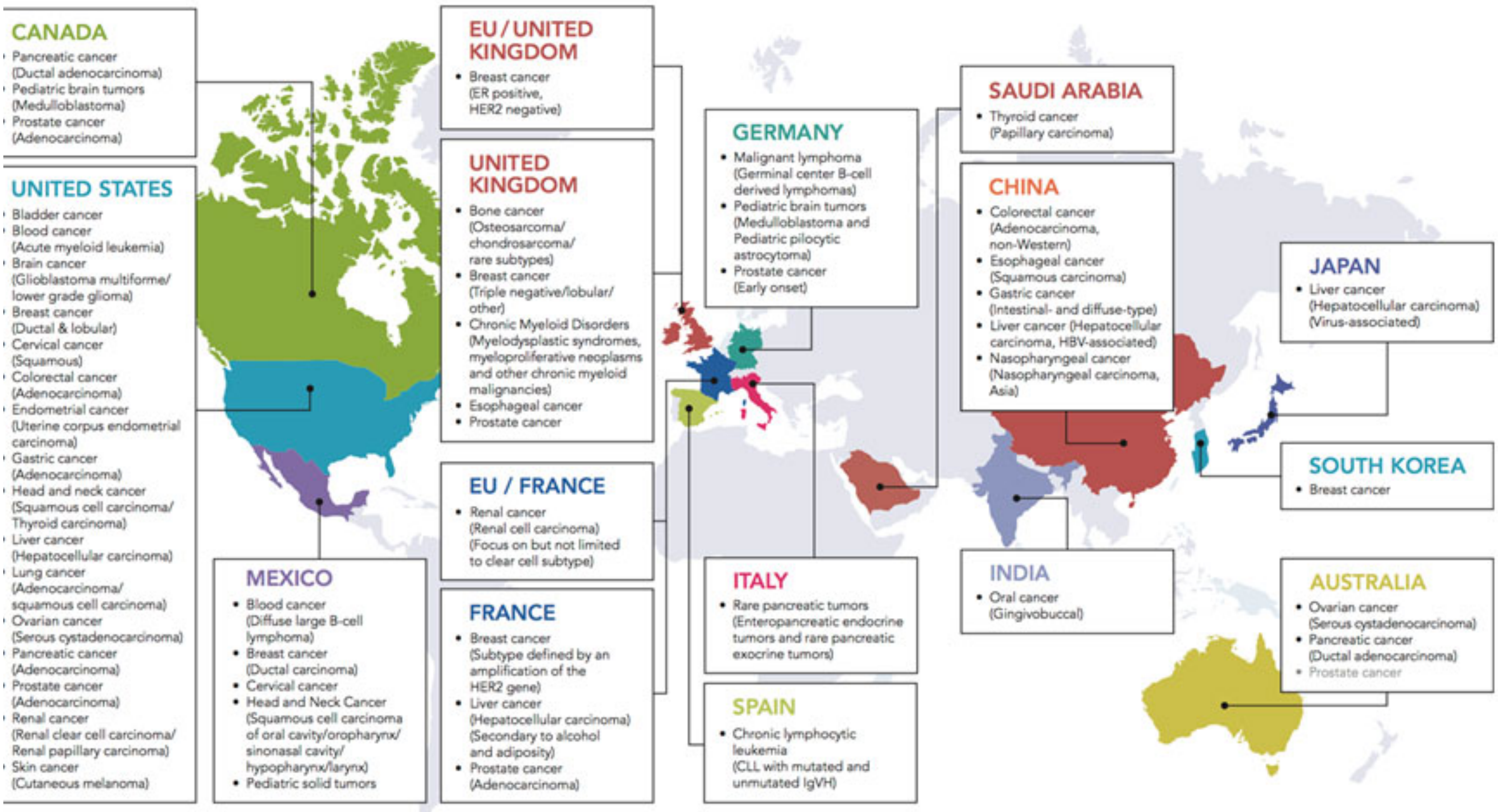
Cancer Genomics Projects Worldwide



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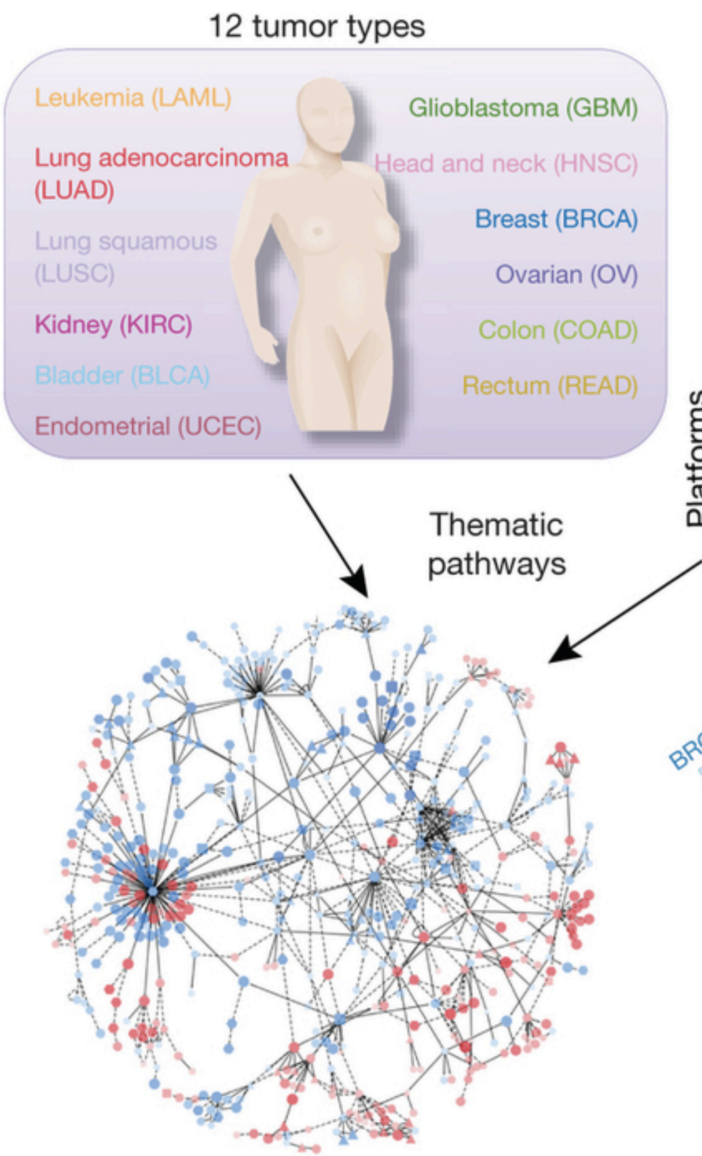
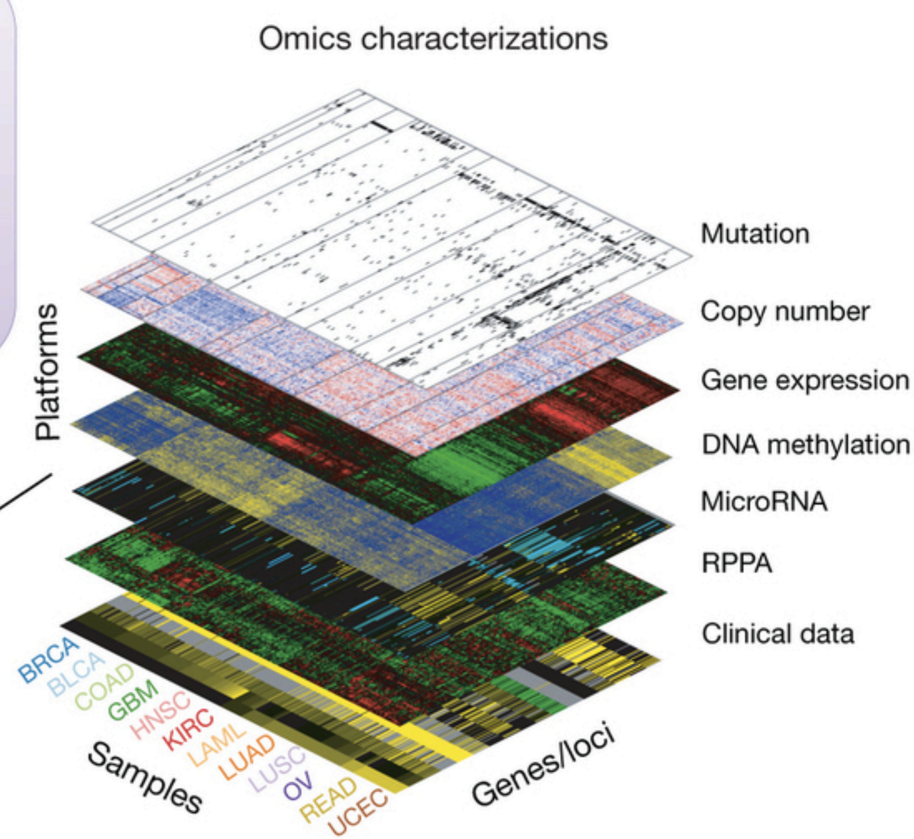
R.K.Wilson 2016

Impact of Large-Scale Cancer Genomics

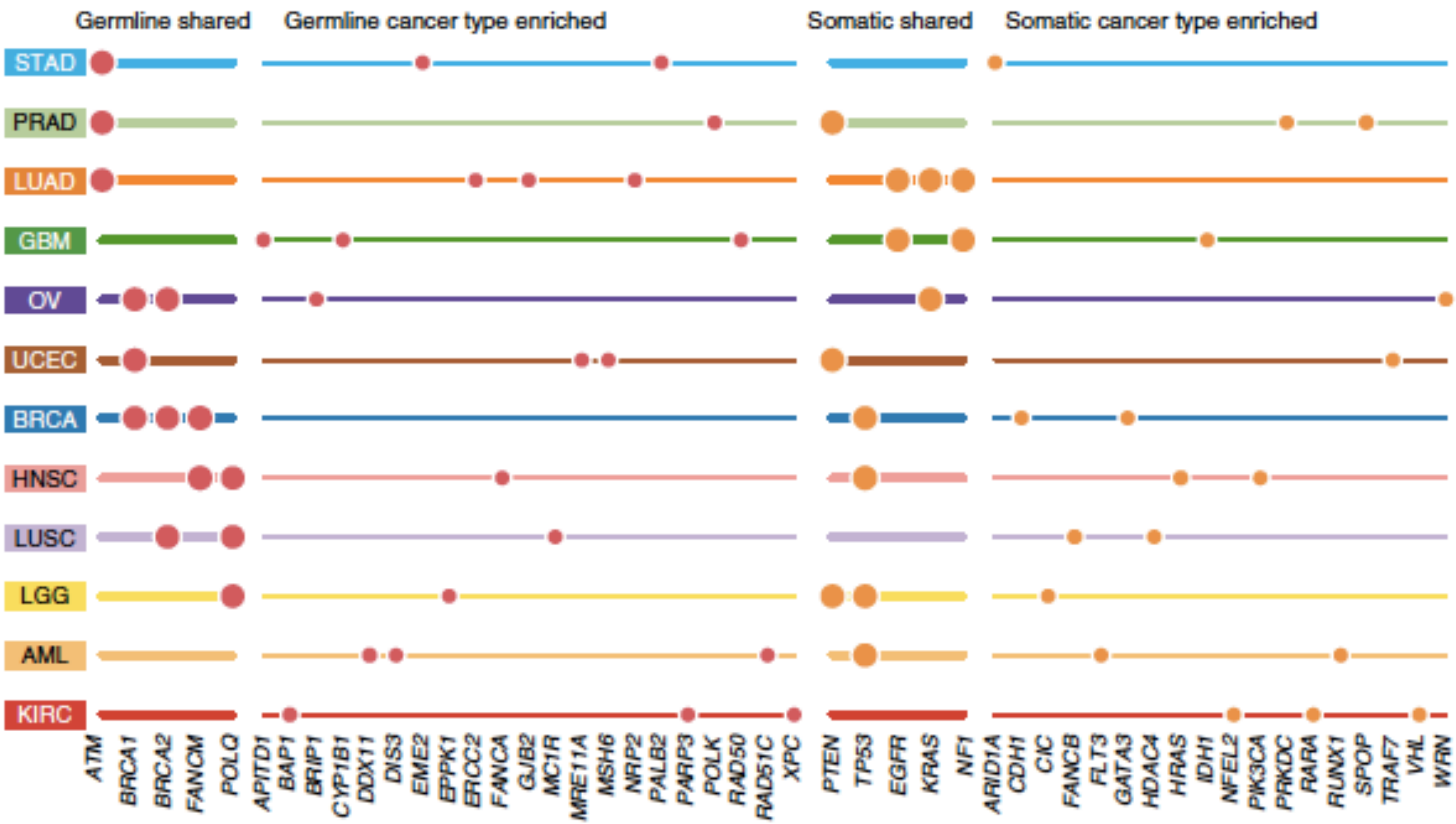




- Cancer genes are shared across tissue sites
- Cancer genes are altered in many ways
- Specific cellular pathways are perturbed by the combined somatic and germline alterations

Nature Genetics 45: 1113-1120 (2013)

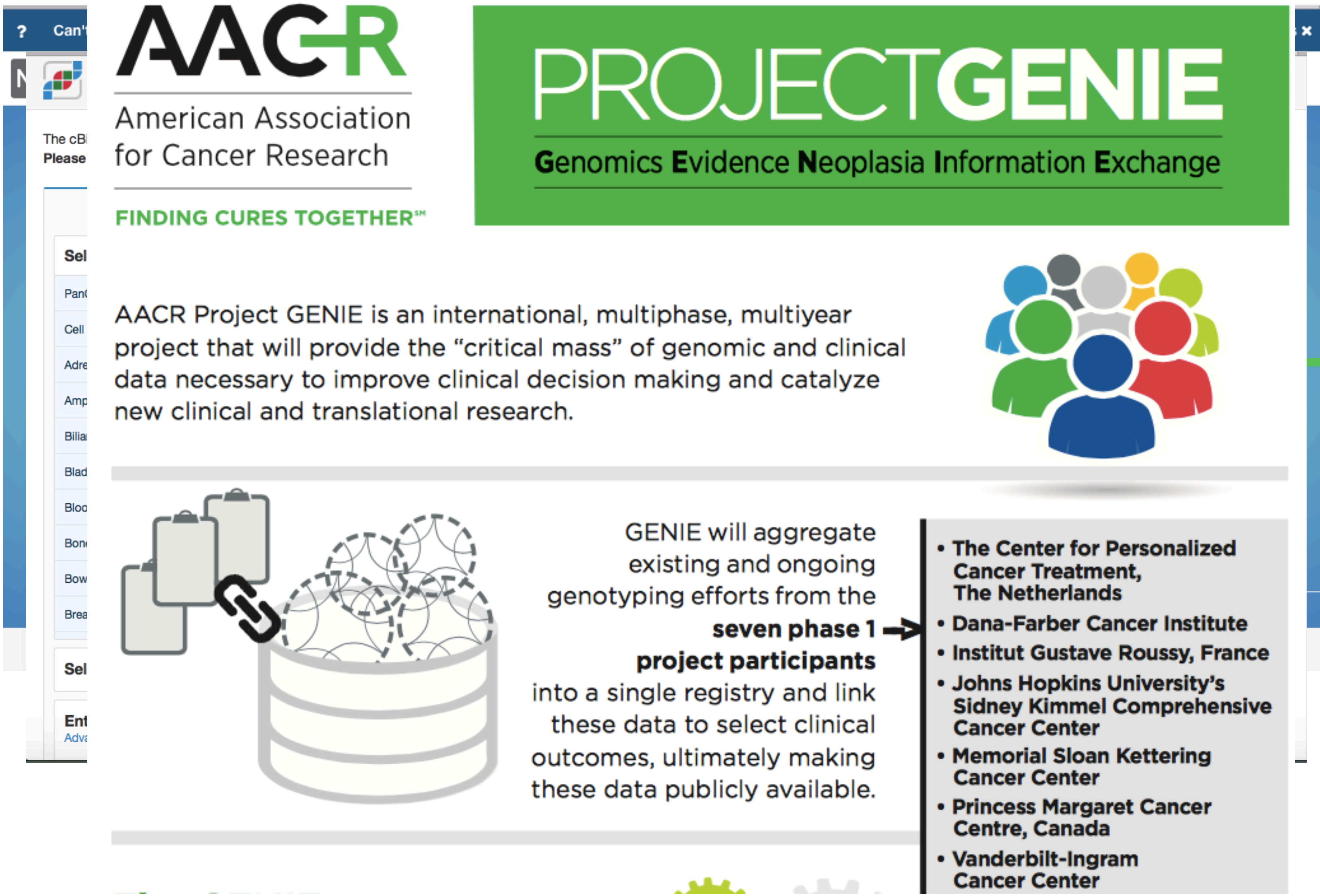
Shared Germline and Somatic Cancer Genes



- 12 TCGA projects: 4,034 cases
- Frequency of rare germline truncations in 114 cancer-susceptibility genes varies widely, from 4% (AML) to 19% (OV), and is notably high in stomach cancer (11%)

C. Lu et al., Nat. Comm. 2015

Big Science = Big Data Resources



Cancer Genomics

Applying Discovery to Clinical Translation

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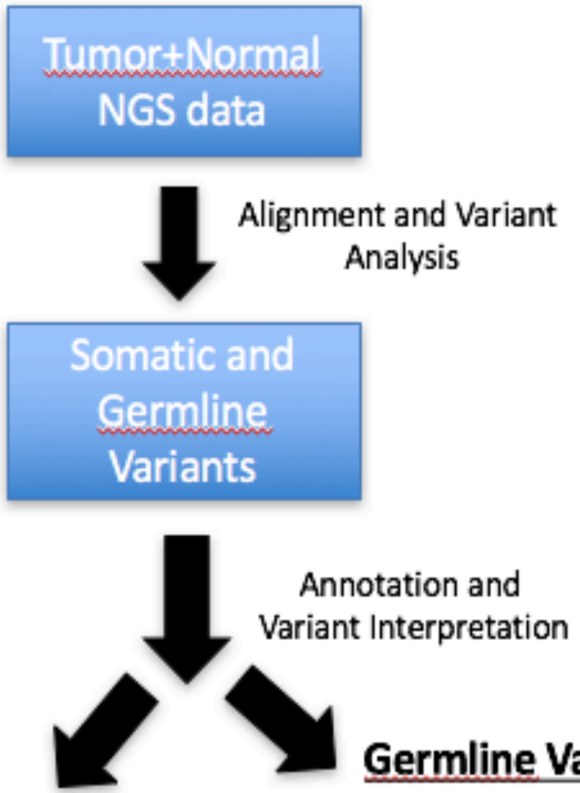
Emerging Paradigm: Informed Clinical Trials

- Big data mining can inform the next generation of targeted therapy trials by enabling the examination of:
  - Tissue sites that predominate in alterations of the target gene/protein driver
  - Co-occurrence and mutual exclusivity of other gene alterations
  - Pathway level evaluation of the altered gene/protein impact in a specific type or subtype of malignancy
- The resulting trials should have enhanced accrual and efficacy

Clinical Applications of Cancer Genomics



Somatic Variants:

- Pathogenic driver mutations (targeted therapy)
- Gene fusion drivers (targeted therapy)
- Amplified cancer driver genes (targeted therapy)

Germline Variants:

- Pathogenic cancer susceptibility mutations (Genetic counseling)

The challenge of NGS is we do not understand the impact of every variant!!

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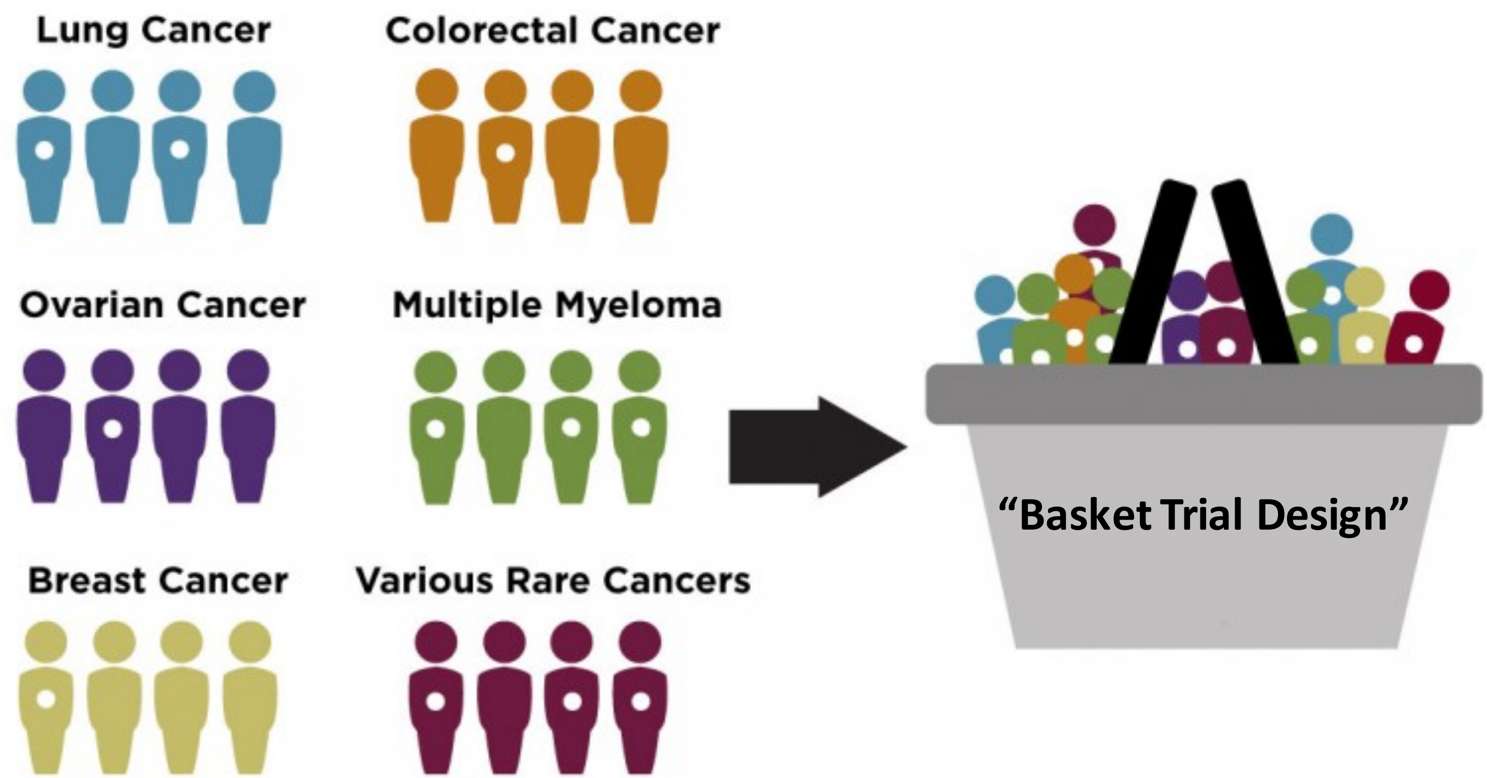
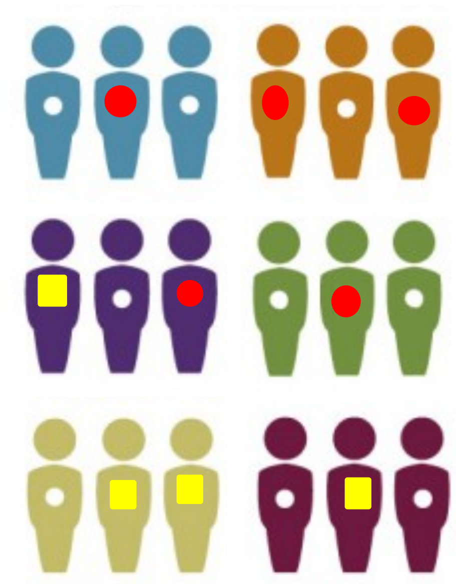
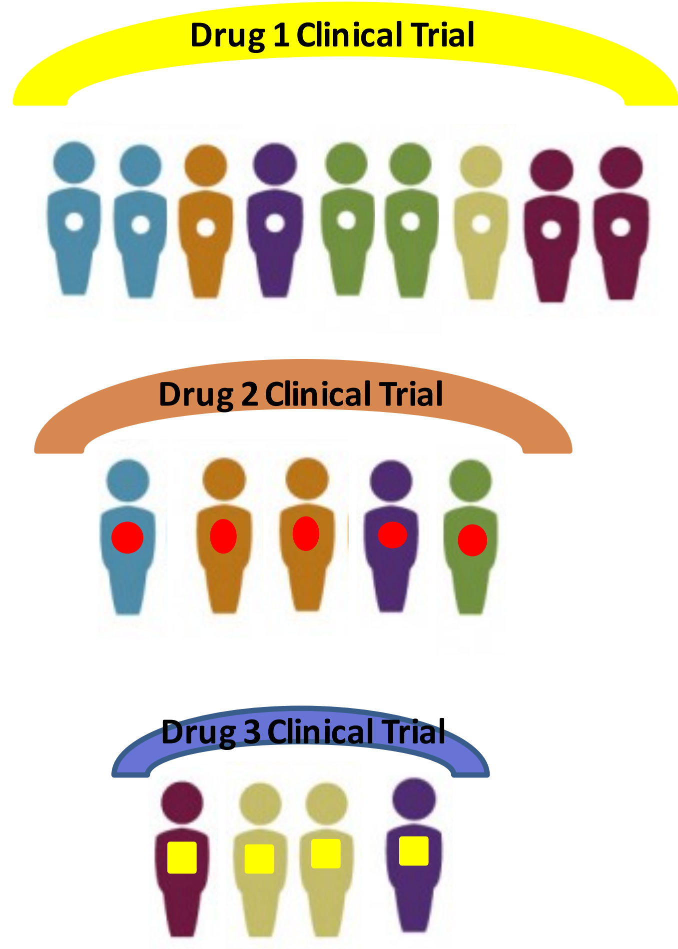
Targeted Therapy Clinical Trial Design: Basket



Targeted Therapy Clinical Trial Design: Umbrella



Different tissue sites
Different target gene drivers



"Umbrella Trial" Design

ARTICLE

doi:10.1038/nature25475

HER kinase inhibition in patients with HER2- and HER3-mutant cancers

David M. Hyman[1], Sarina A. Piha-Paul[2], Helen Won[1], Jordi Rodon[3], Cristina Saura[3], Geoffrey I. Shapiro[4], Dejan Juric[5], David I. Quinn[6], Victor Moreno[7], Bernard Doger[7], Ingrid A. Mayer[8], Valentina Boni[9], Emiliano Calvo[9], Sherene Loi[10], Albert C. Lockhart[11], Joseph P. Erinjeri[1], Maurizio Scaltriti[1], Gary A. Ulaner[1], Juber Patel[1], Jiabin Tang[1], Hannah Beer[1], S. Duygu Selcuklu[1], Aphrothiti J. Hanrahan[1], Nancy Bouvier[1], Myra Melcer[1], Rajmohan Murali[1], Alison M. Schram[1], Lillian M. Smyth[1], Komal Jhaveri[1], Bob T. Li[1], Alexander Drilon[1], James J. Harding[1], Gopa Iyer[1], Barry S. Taylor[1], Michael F. Berger[1], Richard E. Cutler Jr[12], Feng Xu[12], Anna Butturini[12], Lisa D. Eli[12], Grace Mann[12], Cynthia Farrell[12], Alshad S. Lalani[12], Richard P. Bryce[12], Carlos L. Arteaga[8], Funda Meric-Bernstam[2], José Baselga[1] & David B. Solit[1]

- Mutations in HER2 and HER3 kinases have been identified in many human cancer types, including breast, lung, colorectal, bladder
- This report in Nature describes the results of the SUMMIT “basket” trial of neratinib, a pan-HER kinase inhibitor
- Enrolment of 141 patients, 125/HER2 and 16 with HER3 mutations, 21 unique cancer types
- Response to therapy involves the **specific** mutation, the **tissue** of origin, and the **genomic context** of the mutation.

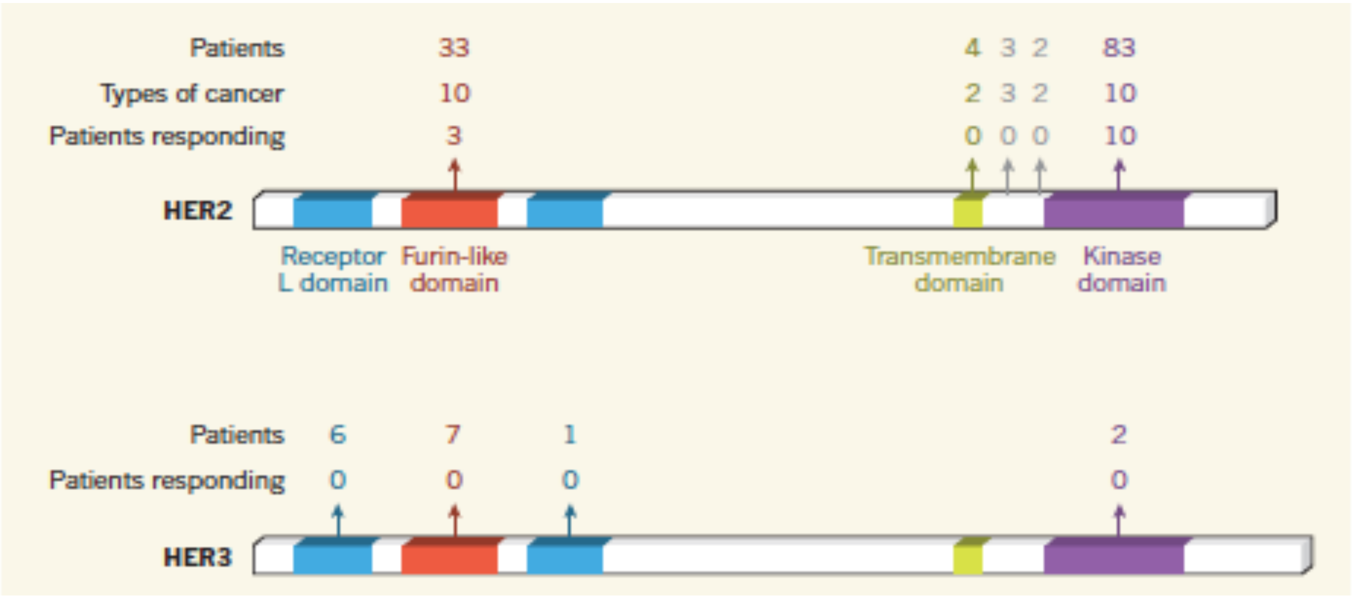
NEWS & VIEWS

CANCER RESEARCH

Many mutations in one clinical-trial basket

If abnormality in a gene is linked to cancer and a drug targets the encoded protein, how can the patients who will respond to the drug be identified if the gene is mutated in many different ways in many different cancers?

ELAINE R. MARDIS



ERSITY

Genomics of Cancer Susceptibility

Identifying Inherited Cancer Risk

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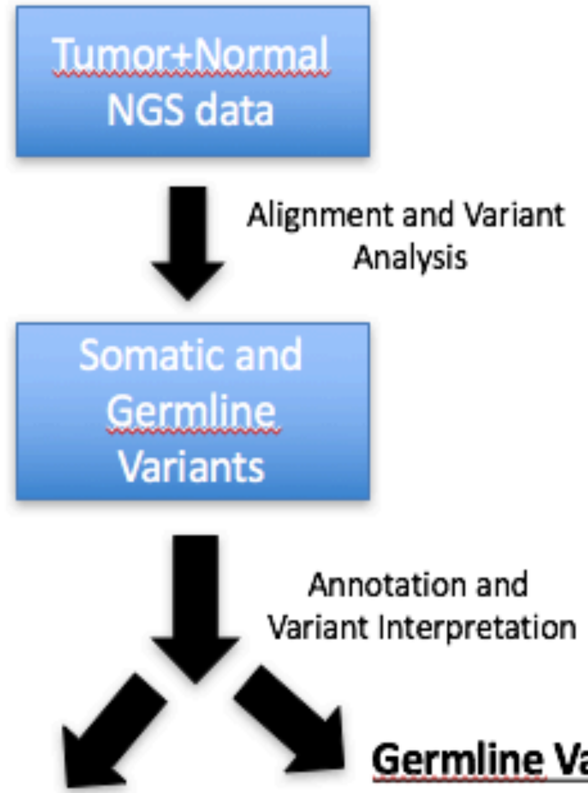
Clinical Applications of Cancer Genomics



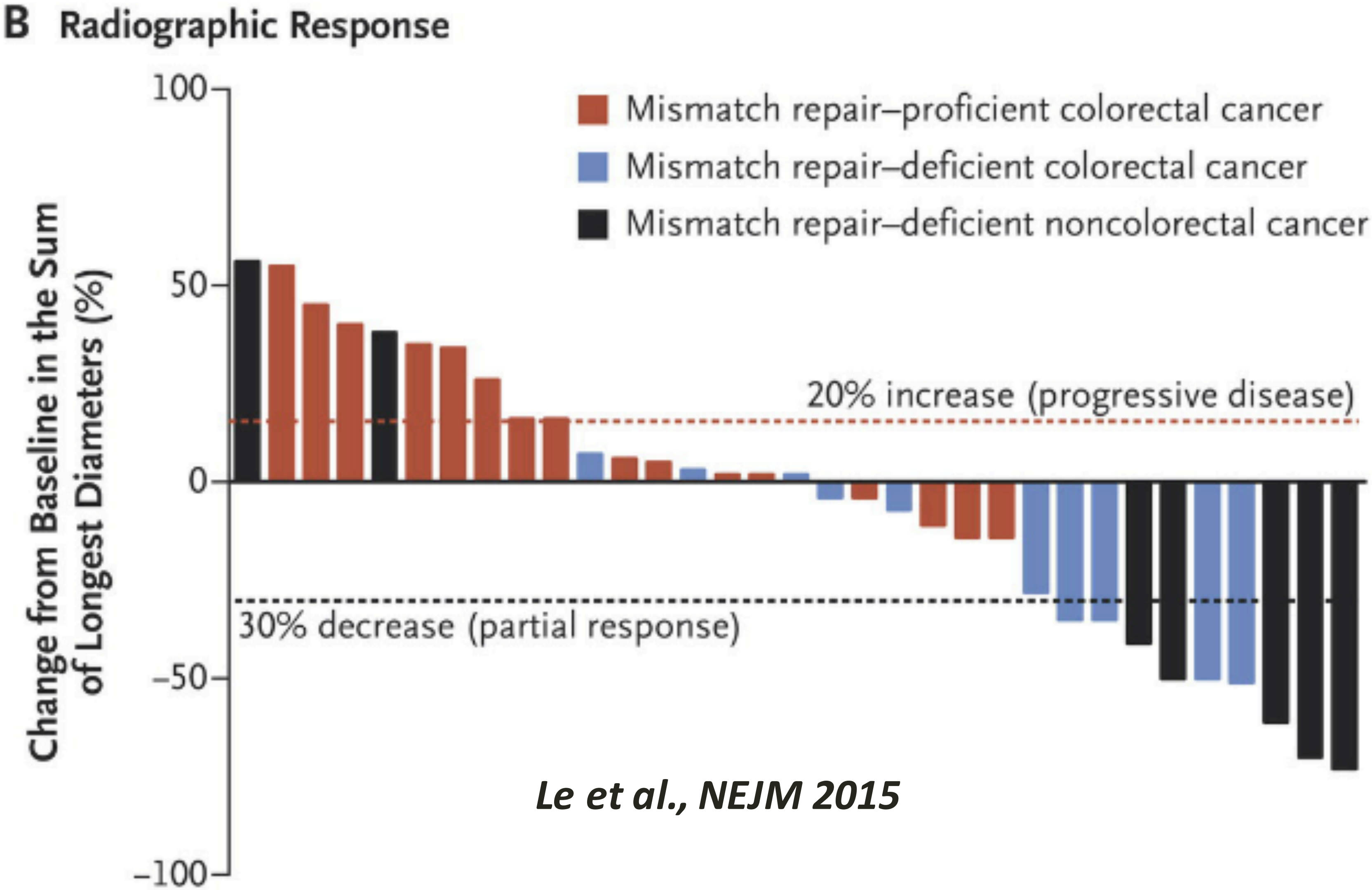
Somatic Variants:

- Pathogenic driver mutations (targeted therapy)
- Gene fusion drivers (targeted therapy)
- Amplified cancer driver genes (targeted therapy)

Germline Variants:

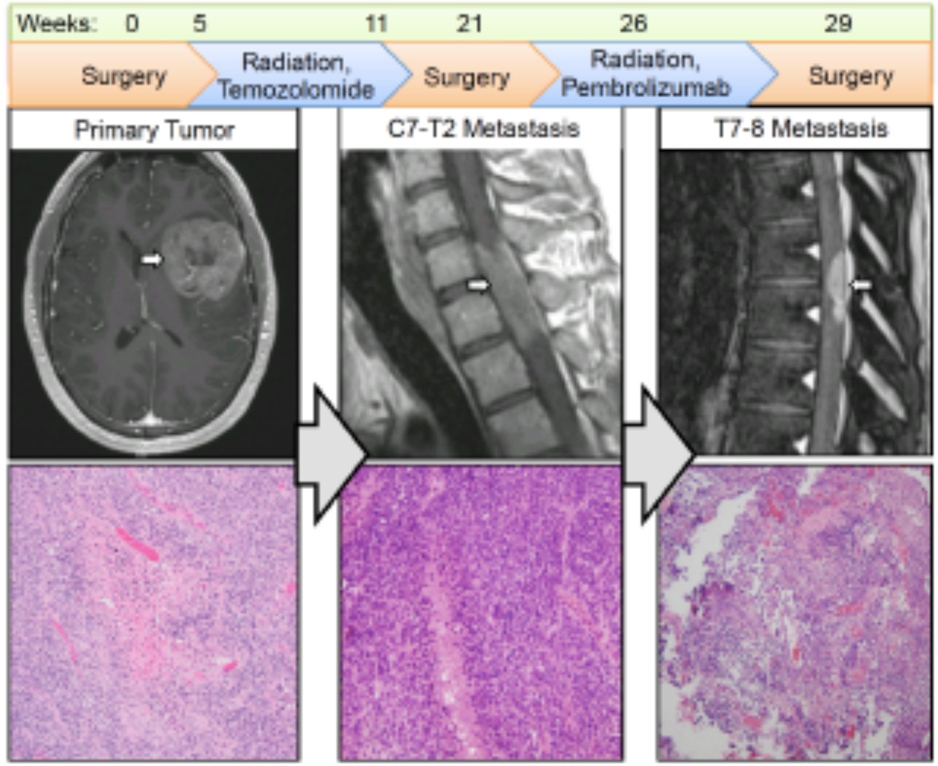
- Pathogenic cancer susceptibility mutations (Genetic counseling)
- Microsatellite instability (Immunotherapy)
- BRCA1/2, other HRD genes (PARP inhibitors)
- Pol E (treatment considerations)

Mismatch Repair Defects and Checkpoint Blockade Response



US FDA approved Pembrolizumab use in all tumor types with MMR-D

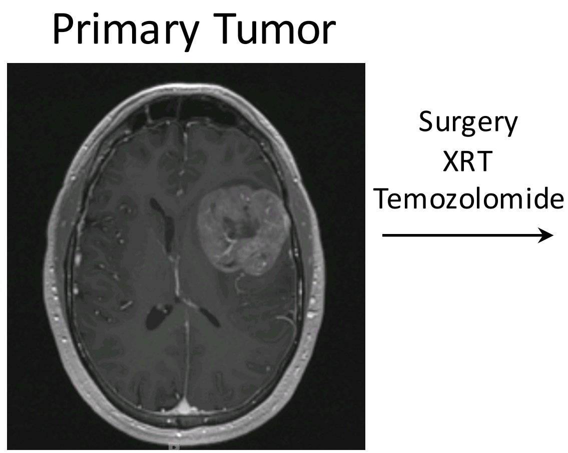
GBM27: Rapid Progression in CNS

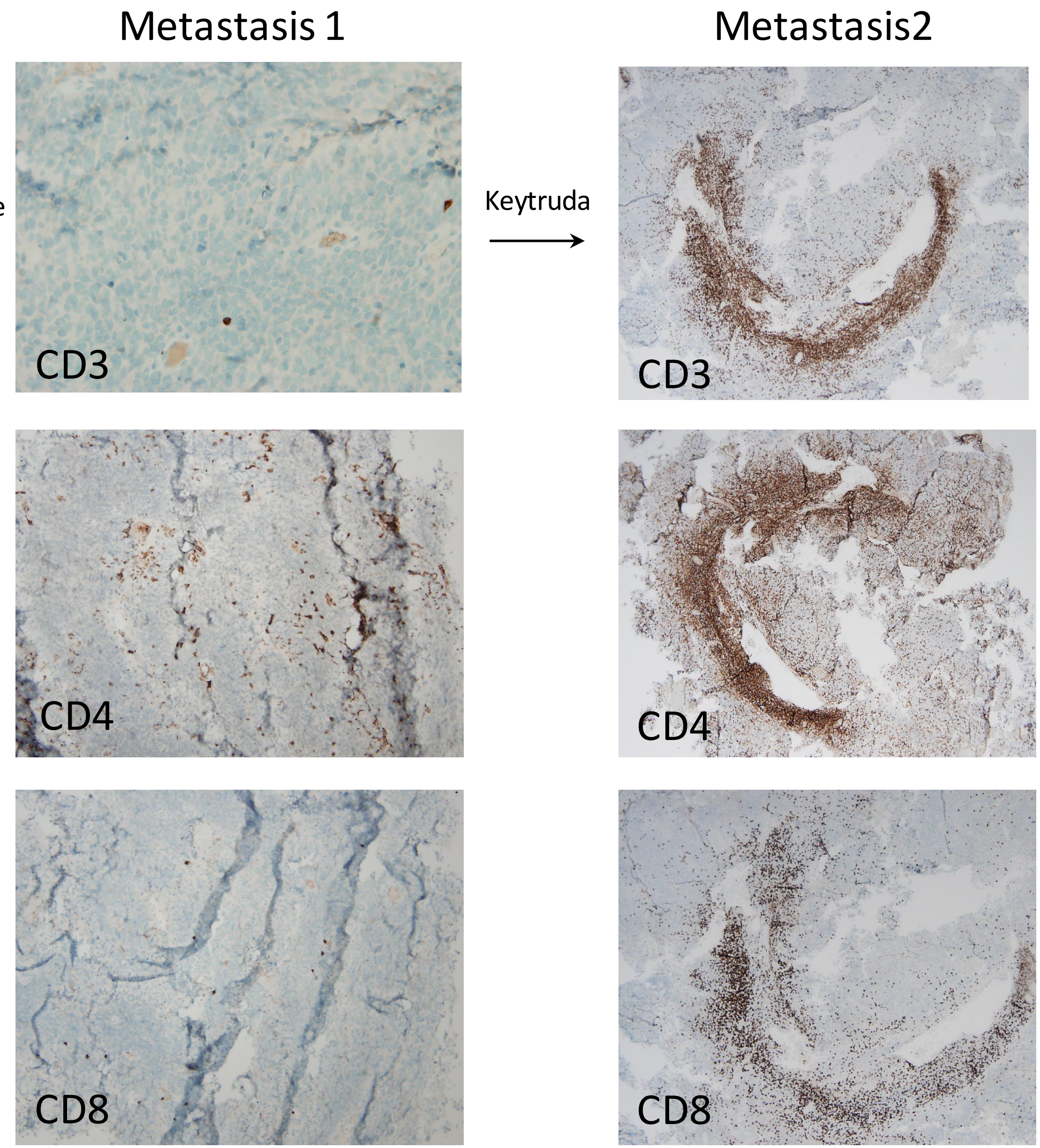


- Male patient, early 30's, prior history of colon polyps
- GBM removed by craniotomy/Post surgery temozolomide and radiation therapy; FMI test indicated high mutation load/pol E mutated germline status
- Spinal metastasis detected after 3 months: treatment with Pebrolizumab
- Second spinal metastasis identified upon complications, removed post-Pembro
- All tumors studied by high coverage exome sequencing compared to PBMC normal, and by IHC

Johanns et al., Cancer Discovery 2016

GBM27: Evolving Immune Response



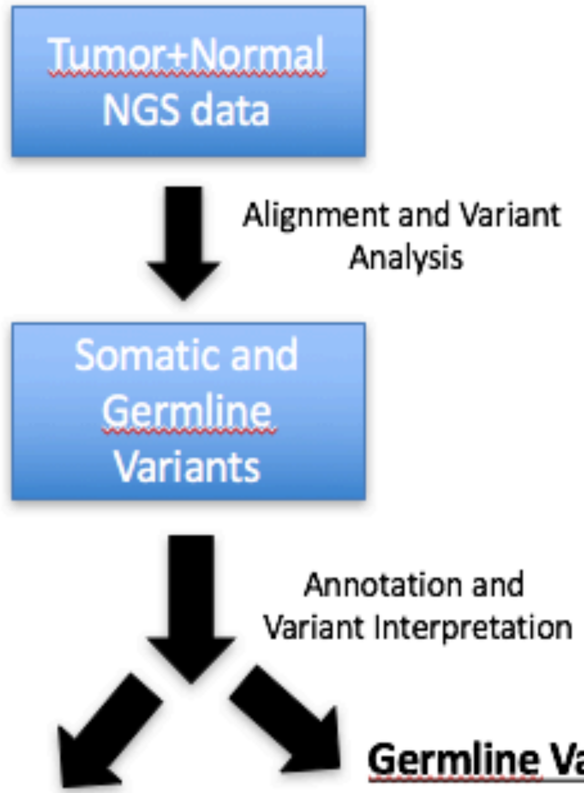


- IHC stains for different immune molecules indicates the treatment with anti PD-1 therapy has resulting in the influx of multiple T-cell types into the second spinal metastasis

Brain Cancer…

…has now surpassed leukemia as the leading cause of cancer-related death in kids

Clinical Applications of Cancer Genomics



Somatic Variants:

- Pathogenic driver mutations (targeted therapy)
- Gene fusion drivers (targeted therapy)
- Amplified cancer driver genes (targeted therapy)
- Calculate mutational load (immunotherapy)
- Neoantigen prediction (immunotherapy incl. vaccine)

Germline Variants:

- Pathogenic cancer susceptibility mutations (Genetic counseling)
- Microsatellite instability (Immunotherapy)
- BRCA1/2, other HRD genes (PARP inhibitors)
- Pol E (treatment considerations)

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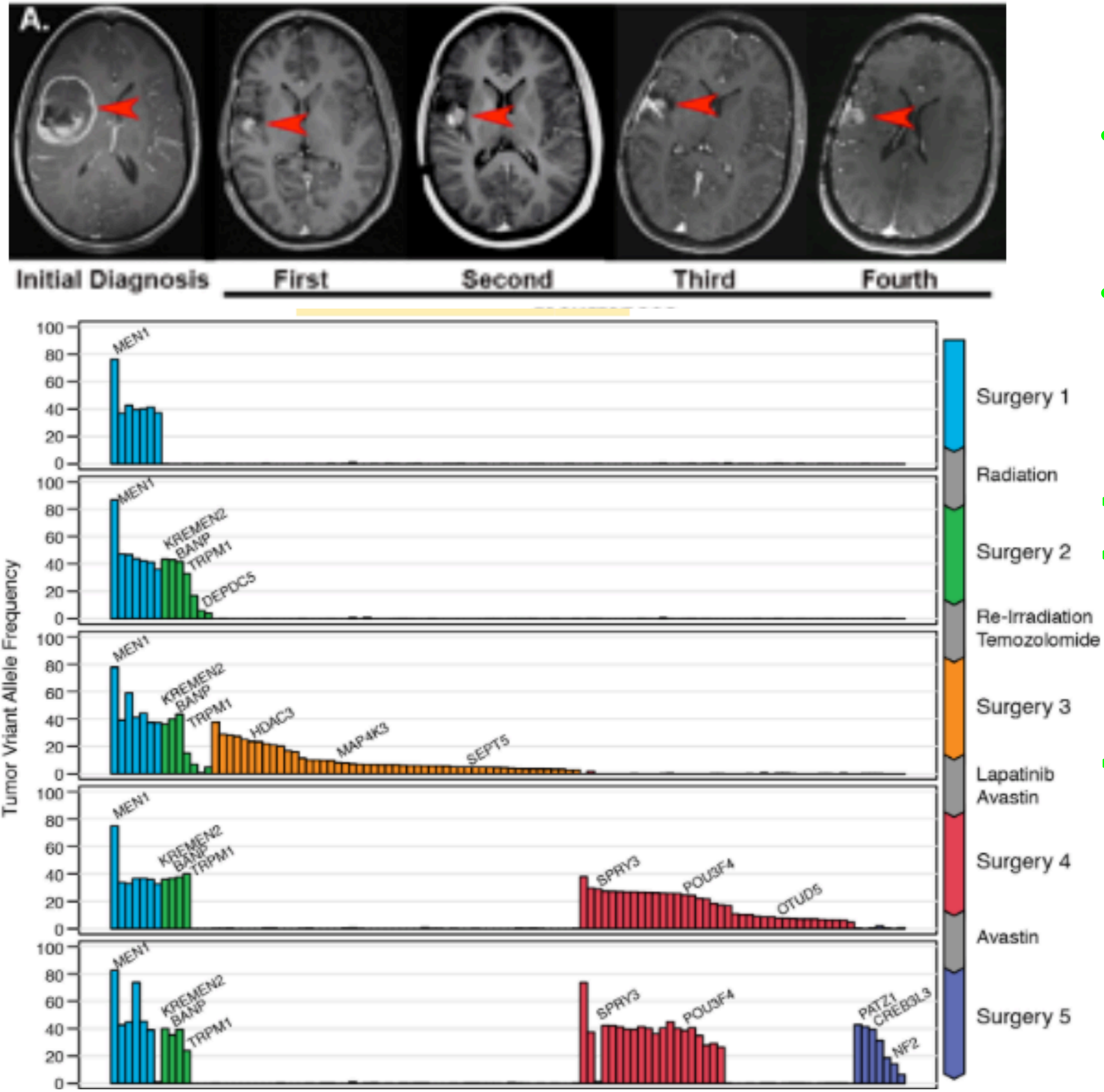
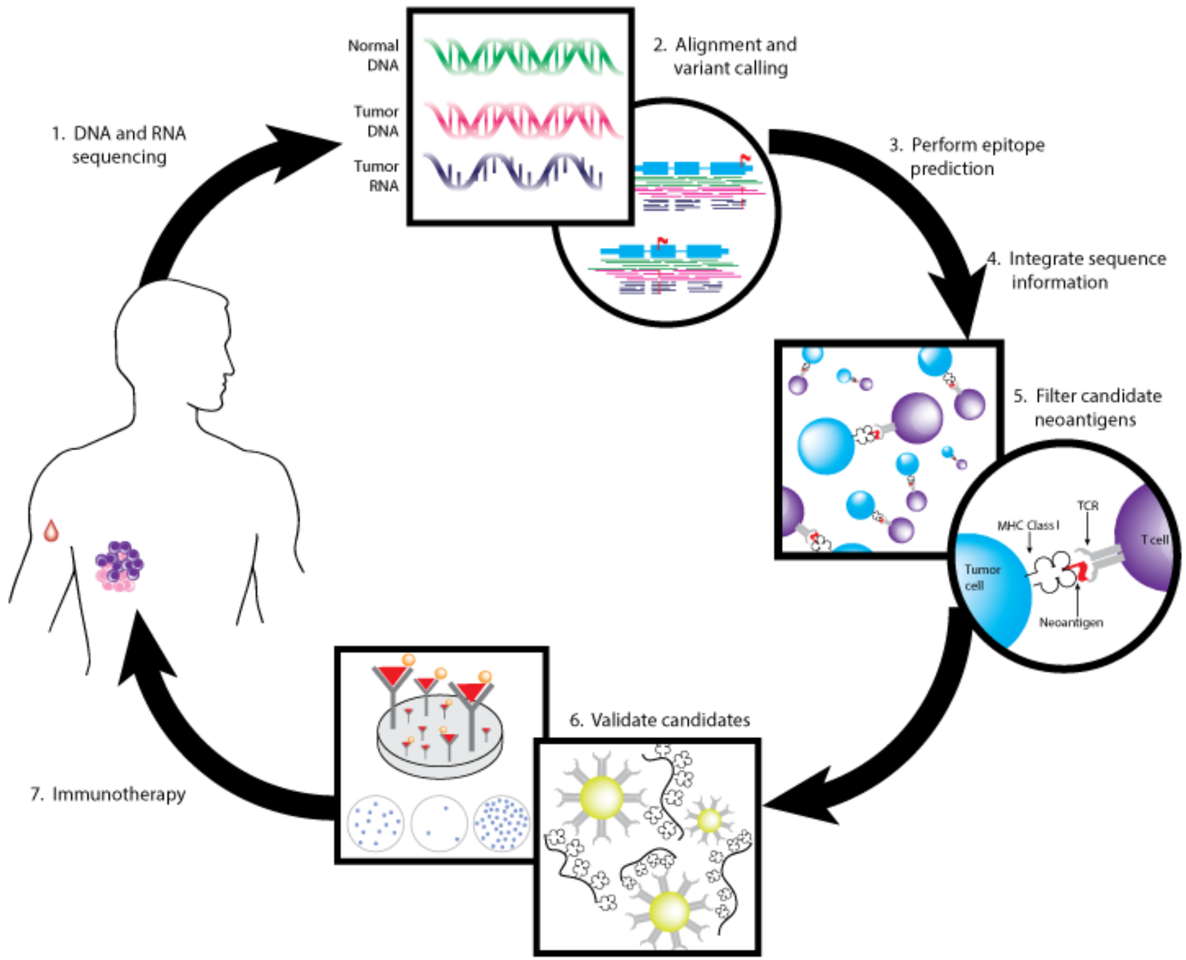
Victoria: Our Inspiration



- Banked resections from a single, pediatric ependymoma patient initially diagnosed early teens
- Sequencing combined 30-fold WGS with 600-fold exome and RNAseq from each sample/timepoint
- Treatment history is known
- Genomic analysis illustrates therapeutic "response" based on increasing clonal complexity over time
- Patient recently had a small remaining tumor mass removed after it began to progress. The tumor was sequenced and analyzed as shown in the lower panel.

C.A. Miller et al., Molecular Case Studies 2018

Genome-guided Cancer Vaccine Design

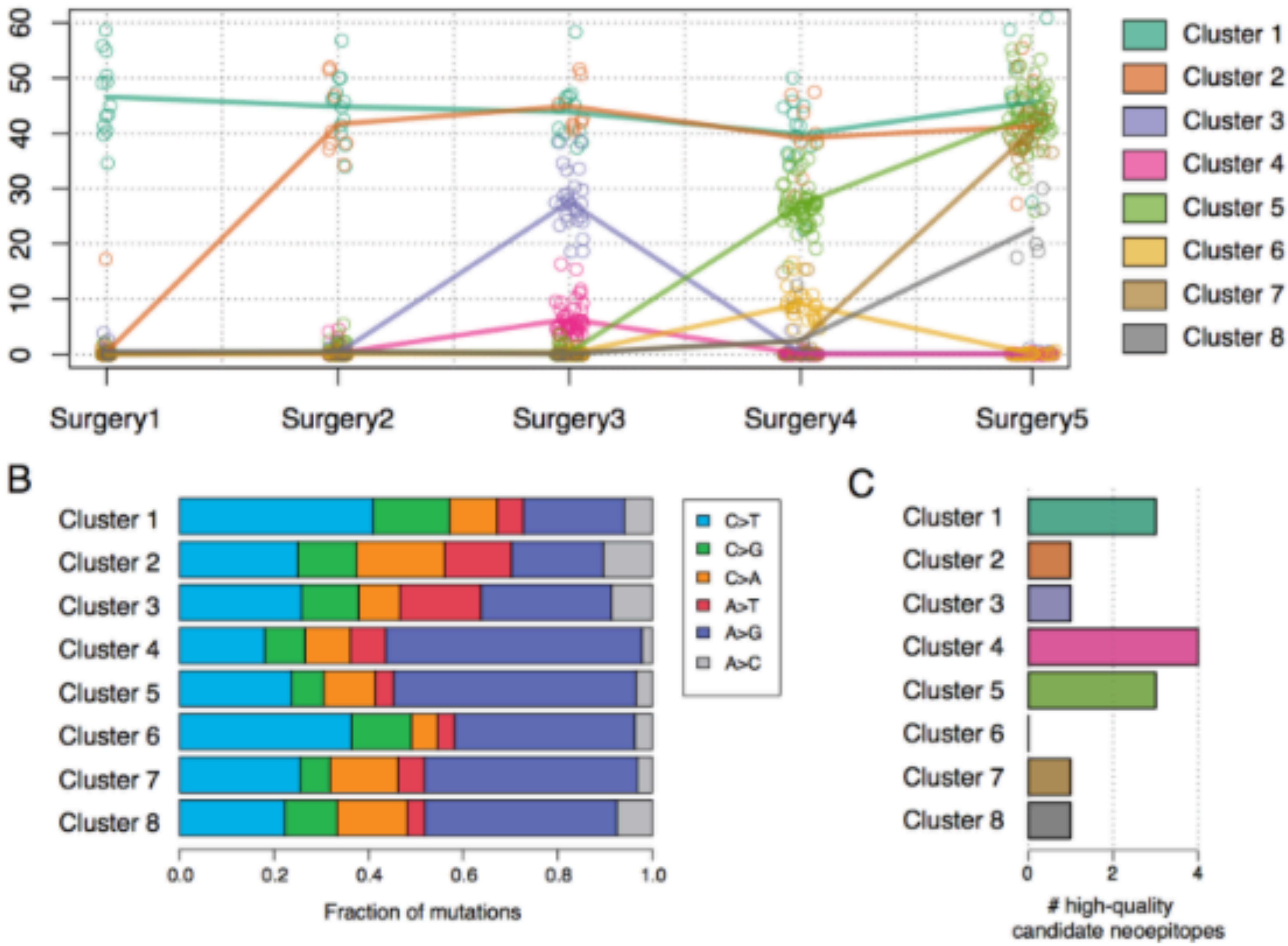


Cancer cells make abnormal proteins due to mutations that change amino acid sequences

We predict the amino acid changes from NGS analysis, then predict which peptides will be ”neo”antigens, based on the HLA types in the patient’s genome

The most antigenic peptides can be used to design a patient-specific vaccine that will elicit an immune response to the tumor which is highly specific and has few side effects

Victoria: Neoantigen Predictions



A DNA minicassette vaccine, designed with 10 neoantigen sequences (9 class I, 1 class II), was synthesized for this patient. She has had a partial response in her recurrent tumor.

Pediatric Brain Cancer Initiative

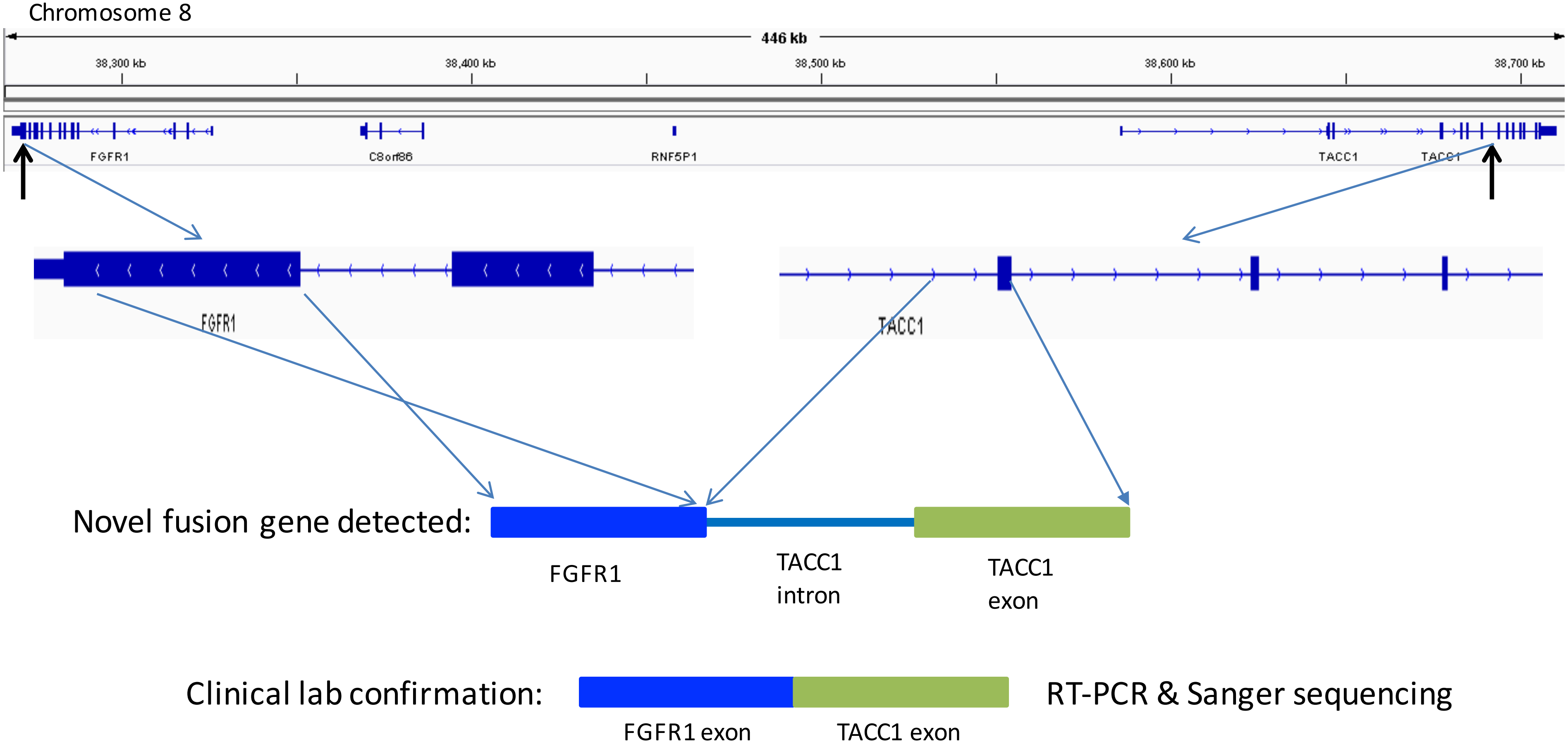
Collaborative effort at Nationwide Children's Hospital to identify pediatric brain cancer cases with indeterminate diagnoses or recurrent disease and study these patients by NGS-based analyses

- compare tumor to normal DNA for evidence of mutations in genes that might indicate a therapy
- sequence and characterize RNA from the tumor to aid in molecular subtyping
- our first case was identified end of April 2017
- infant male pt. presenting with seizures
- brainstem tumor based on MRI was resected
- unclear differential diagnosis by pathology

Nolan: Differential Diagnosis

The differential diagnosis for this primary CNS neuroepithelial neoplasm includes the differential of the so- called *Oligodendroglioma-LIKE* small round cell with clear cytoplasm tumors: Extraventricular Neurocytoma/ neurocytic neoplasm (slightly favored based on current IHC panel results as described below and general morphology, with calcification, although together with smear/ squash prep morphology seems even more suspicious for an atypical Mixed Glio-Neuronal/ Neurocytic neoplasm); vs. less likely Clear cell/ Vascular Ependymoma (plausible given observed anatomic distribution, patient age, nuclear clustering and calcification, *but* GFAP is minimally reactive, rosettes are overall relatively inconspicuous, EMA is completely negative including for any dots or rings, ***and*** Olig-2 is partly positive with small round tumor cells, the latter all militating against this possibility); Oligodendroglioma (ODG: would be highly unusual and very unexpected at this very young age and most typically is *non*-enhancing, but morphology including calcification, palisading nuclei and nuclear clustering, anatomic distribution, S100++/ GFAP minimal+, could be consistent with ODG, *although* Olig-2 staining would be *expected* to be seen in an even higher percentage of the small round tumor cells than appreciated herein); Pilocytic Astrocytoma/ Ganglioglioma (PA/ GG; not biphasic or classic nor great fit on morphology except that ODG- like components are well known in PA, nuclear clustering and nuclear palisading are also better known components of this entity than most the other possibilities except for ODG, which would be as noted above, very unusual); Dysembryoplastic Neuroepithelial Tumor (DNT; morphology is not classic herein but could be atypical/ early/ variant form and IHC staining pattern also fits this entity in general, with S100++, GFAP minimal, Olig-2 partial+, Ki-67 intermediate, and occasional foci of more discrete nodularity seen, although calcification as clearly seen herein is believed to be relatively unusual or uncommon in DNT). INI-1 IHC, given patient age, sex, and the highly variable morphologies reported therein, **is pending at OSU** (we are out of stock) to exclude the relatively unlikely possibility of an AT/ RT (Atypical Teratoid/

Nolan: An FGFR1:TACC1 Fusion

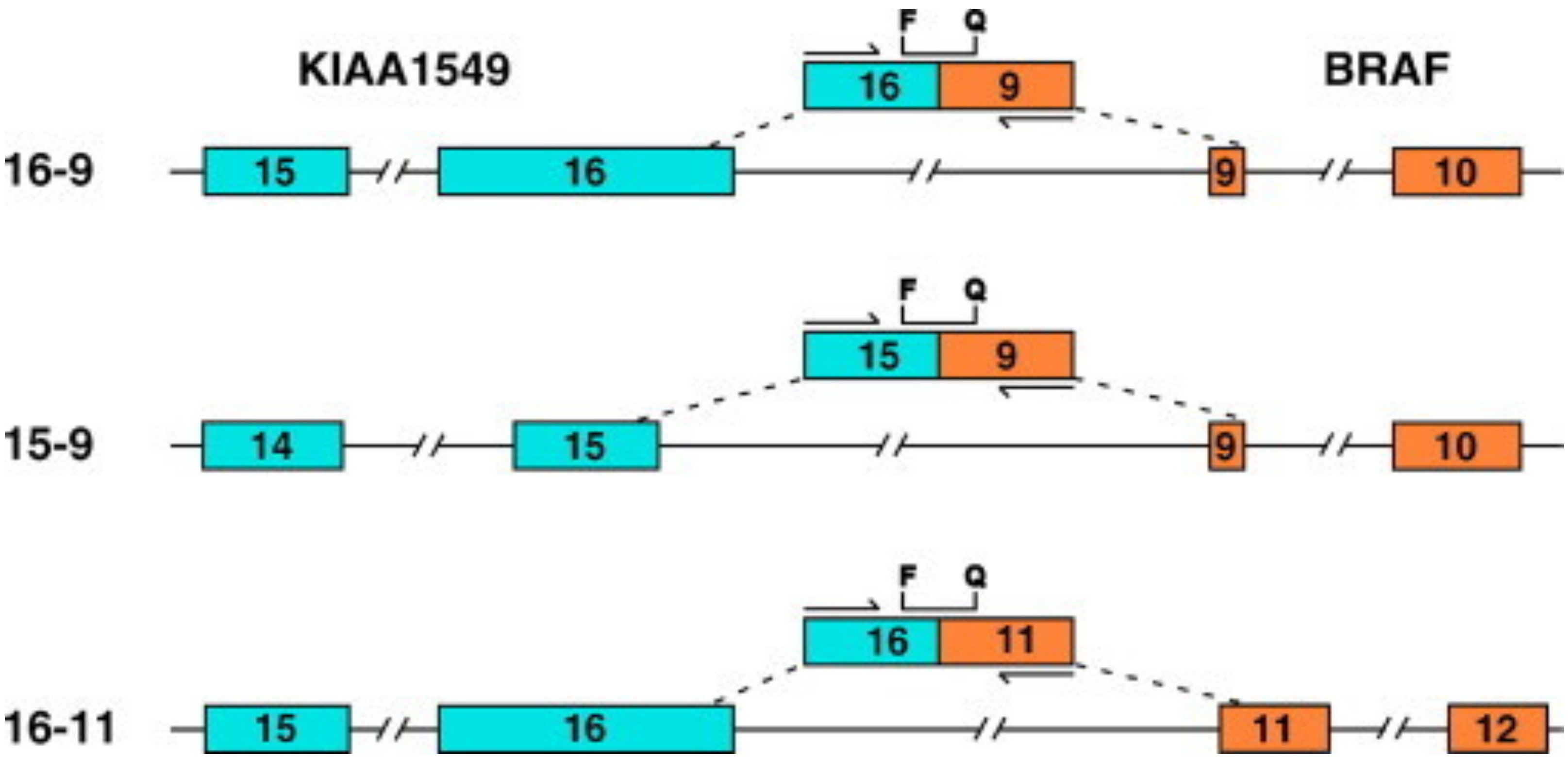


- A FGFR1:TACC1 fusion on Chr. 8 is present and expressed in the patient's tumor RNA
- Similar fusion genes previously observed in pediatric & adult brain tumors
- Potentially TARGETABLE using FGFR inhibitors

Tess: Pilocytic Astrocytoma

- Female patient, pilocytic astrocytoma
- Germline ALK mutation (pGly1121Asp); pathogenic
- Somatic mutation in MAEL (pAla33Val): cancer testis antigen
- CNV analysis revealed: complete loss of one copy each Chr. 1, 2, 4, 13, 16, 19, 22; complete gain 1 copy of Chr. 6, small interstitial gain Chr 7
- Structural variant analysis revealed…

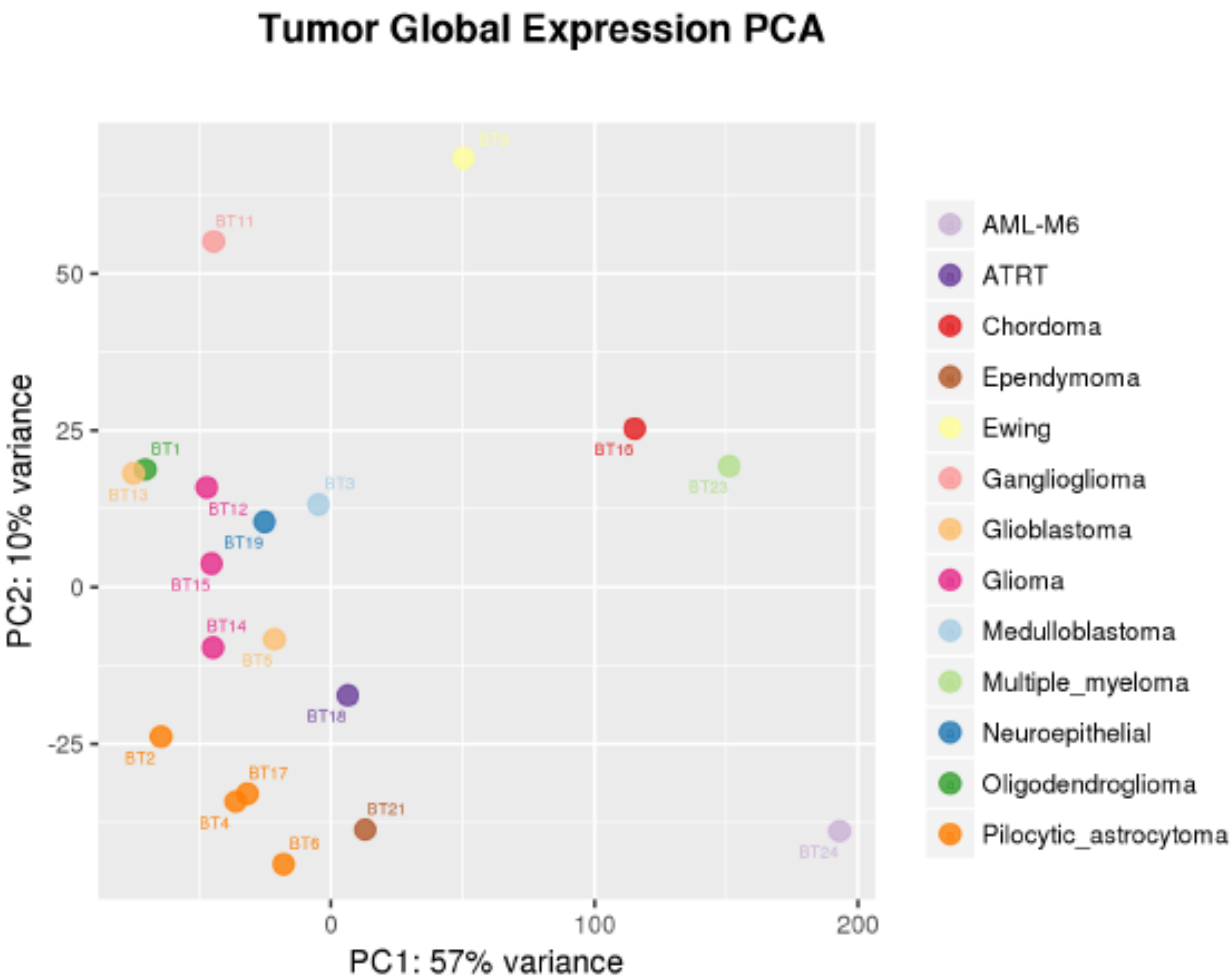
Tess: Somatic KIAA1549^{15}-BRAF^{9} fusion



This patient appears to have the second of the pictured fusions – fusing exon #15 of KIAA1549 with exon #9 of BRAF. The KIAA1549-BRAF fusions have been reported in low grade gliomas from large-scale studies of pediatric patients. For this patient, a **MEK** inhibitor is an appropriate therapy.

Image from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3194058/

RNA-based Tumor Subtyping by PCA



NCH Neuro-oncology Genomics: Initial Results

No.	Diagnosis	Age	Primary somatic finding	Primary germline finding
1	Indeterminate, most consistent with oligodendroglioma	1	FGFR1-TACC1 fusion	—
2	Pilocytic astrocytoma	4	KIAA1549-BRAF	—
3	Medulloblastoma, WHO grade IV (Non-WNT, Non-SHH?)	15	CNV loss, including TP53	—
4	Pilocytic Astrocytoma	4	KIAA1549-BRAF	—
5	Glioblastoma (recurrence)	12	PIK3CA (c.1633G>A:p.Glu545Lys)	—
6	Pilocytic Astrocytoma	5	FGFR1 (c.1960A>G:p.Lys654Glu); missense SNP in PTPN11 (c.205G>A:p.Glu69Lys)	—
7	Ewing-like Sarcoma	6	EWSR1-CREB3L3 fusion	—
8	Ganglioglioma, WHO grade 1	10	BRAF (c.1799T>A:p.Val600Glu), previously reported	ATM (c.2062G>A:p.Glu688Lys)
9	Diffuse Midline Glioma, H3 K27M-mutant, WHO grade IV	6	PIK3R1 (c.1690A>G:p.Asn564Asp)	—
10	Indeterminate, high grade glioma/astrocytoma	17	Multiple truncating mutations in ATRX, NF1 (compound het), CBL	PMS2 (c.137G>T:p.Ser46Ile)
11	Ganglioglioma, WHO grade 1	13	BRAF (c.1794_1796dupTAC:p.Thr599dup)	—
12	Glioma (low grade)	8	—	—
13	Clival Chordoma	10	Biallelic loss of CDKN2A and CDKN2B (chr9 copy loss & full gene deletion)	—
14	Pilocytic Astrocytoma	5	KIAA1549-BRAF fusion	—
15	Atypical Teratoid Rhabdoid Tumor	1	Biallelic loss of function of SMARCB1 (chr22 copy loss & c.482delA:p.Asp161fs)	—
16	Neuroepithelial tumor (high grade)	2	—	—
17	Ependymoma	15	High number of CNV changes, further investigation ongoing	—
18	Choroid Plexus Carcinoma, WHO grade 3	4	Hyperhaploidy	TP53 (c.625C>T:p.Arg209Trp), mosaic

Druggable target (n=9)
Likely poor outcome (n=6)
Inconclusive (n=3)

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Future of Genomics-based Diagnostics

- One clear challenge is accelerating the speed of data analysis to identify genomic variants more quickly and accurately
- Data integration from DNA, RNA, protein will provide a more complete picture of the biology of disease
- Expanding our understanding of healthy tissue biology will also provide an important contrast to diseased tissue
- Sharing information and know-how will ensure access to all patients

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Our patients and families!

Et, merci Francis!!

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Merci à tous!

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