The Elizabeth H. and James S. McDonnell III
McDonnell Genome Institute
at Washington University

Genomic data visualization and interpretation

Malachi Griffith, Obi Griffith, Zachary Skidmore

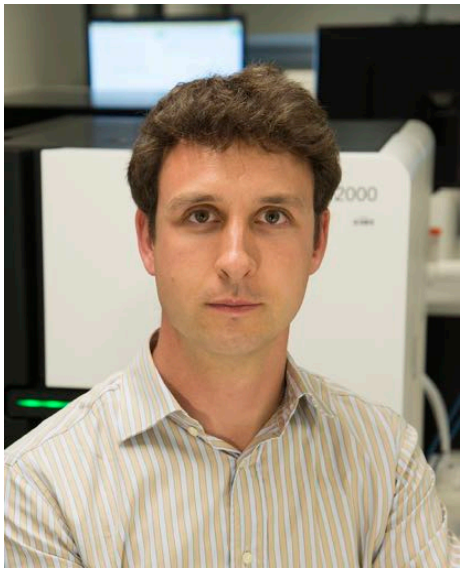
Evomics, Workshop on Genomics

Jan 11, 2019

Český Krumlov, Czech Republic

Introductions to course instructors

Malachi Griffith, PhD
Assistant Professor of Medicine
Assistant Professor of Genetics
Assistant Director, MGI



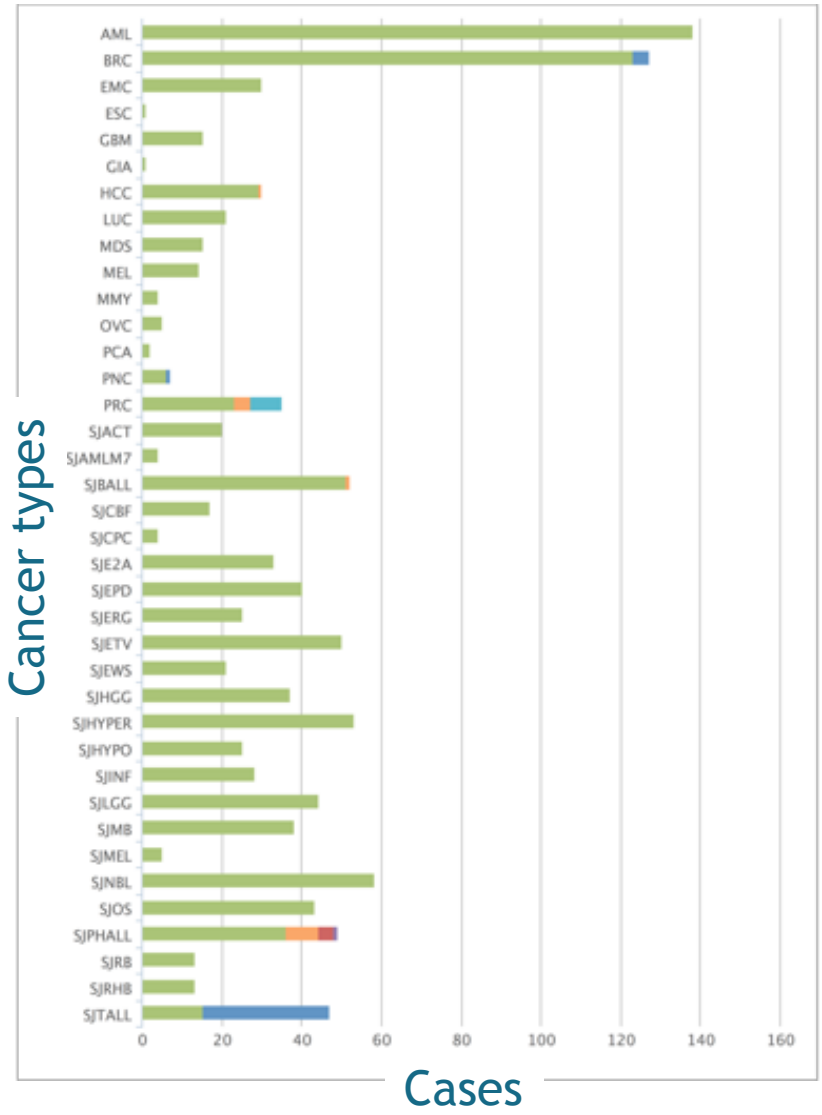
Obi Griffith, PhD
Assistant Professor of Medicine
Assistant Professor of Genetics
Assistant Director, MGI

Zachary Skidmore, MSc
Staff Scientist, MGI

GenVisR creator

McDonnell Genome Institute, Washington University School of Medicine

The McDonnell Genome Institute has pursued the field of genomics since inception: >1000 whole genomes, >5000 exomes, >1000 transcriptomes for dozens of tumor types



MGI – 1000+ tumor whole genomes, many more exomes, X10 & NovaSeq will accelerate this!

- Human Genome Project
- First to sequence and analyze a tumor whole genome sequence (Ley et al, 2008)
- Major contributor to TCGA, PCGP, etc.
- Most comprehensively sequenced single patient tumor ever published (Griffith et al, 2015)
- Early proof-of-principle for cancer precision medicine (Griffith et al, 2016)
- Analysis and tools for first personalized cancer vaccine design in humans (Carreno et al, 2015)
- Many other widely used tools

Overview of lab's research (griffithlab.org)

- **Cancer genome analysis**
  - Breast cancer, Liver cancer, Lung cancer, Head and neck cancer, etc.
  - Variant Interpretation
  - Immunogenomics

- **Precision medicine for cancer**
  - Genomics Tumor Board
  - Case Reports
  - Clinical Trials
  - Personalized Cancer Vaccines

- **Education projects**
  - RNA-seq analysis and cloud computing (CBW, Toronto)
  - Advanced Sequencing Technologies and Applications (CSHL, New York)
  - Genomic Data Visualization/Interpretation (Physalia Courses, Berlin)
  - Precision Medicine Bioinformatics (PR Informatics, Glasgow)
  - High-Throughput Biology: From Sequence to Networks (CSHL / CBW, New York)
  - Workshop on Genomics (Evomics, Český Krumlov)

- **Tool development**

Where tools/resources do not exist we build them

DGIdb
THE DRUG-GENE INTERACTION DATABASE

www.dgidb.org
Search genes for known and potentially druggable interactions

pVAC-Seq

https://github.com/griffithlab/pVAC-Seq
Personalize vaccine design

DoCM
DATABASE OF CANONICAL MUTATIONS

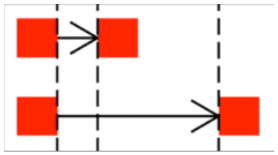
www.docm.info
Filter against highly curated set of mutations known to cause cancer

GenVisR
Bioconductor
OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

https://github.com/griffithlab/GenVisR
Create genomic visualizations

CIViC
CLINICAL INTERPRETATIONS OF VARIANTS IN CANCER

www.civicdb.org
Identify highly curated summaries of clinical interpretations for variants in cancer



regtools

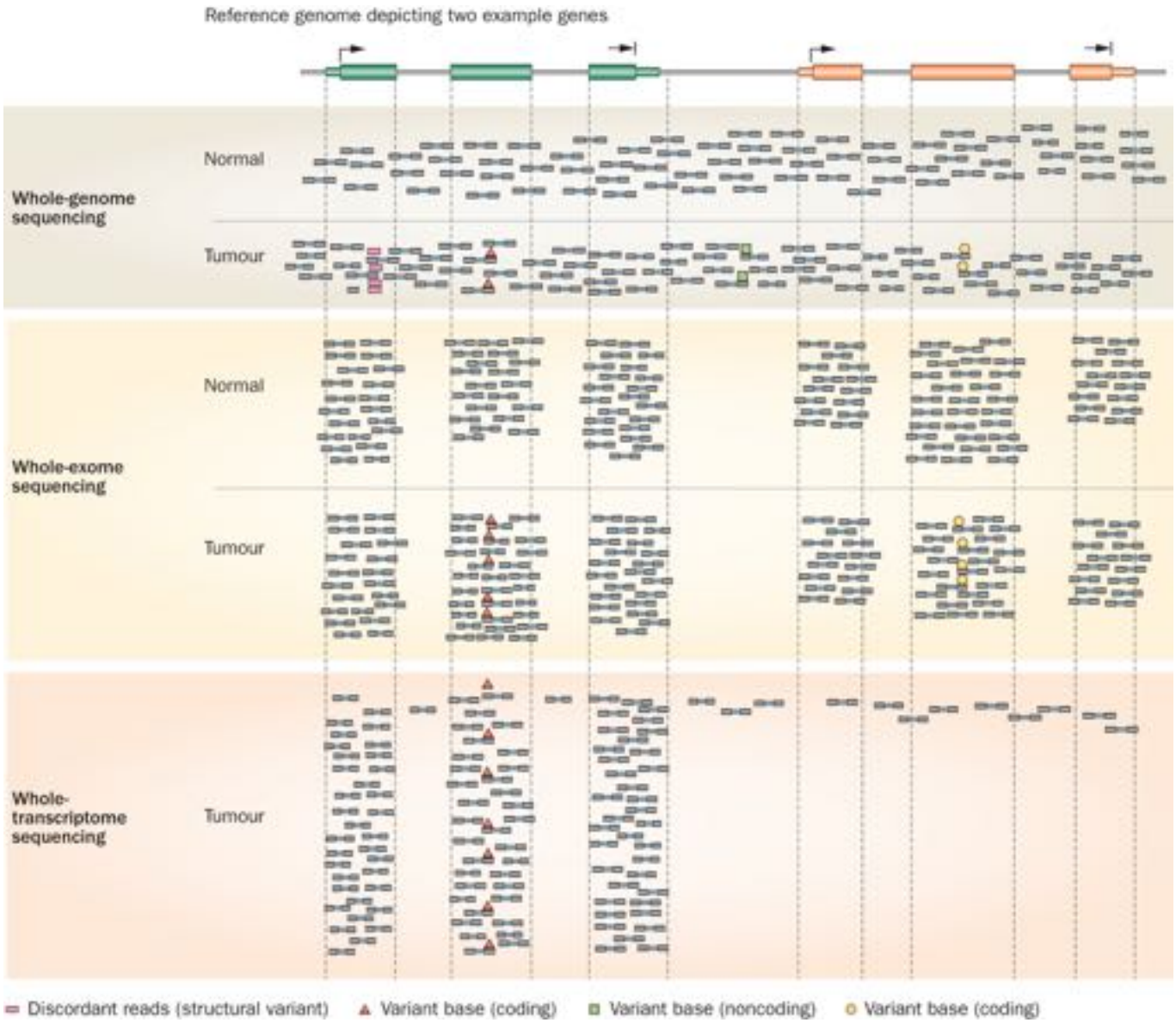
https://github.com/griffithlab/regtools
Identify regulatory variants

Whole genome, exome, transcriptome and other ‘omic’ sequencing allows us to detect and confirm many different variant types

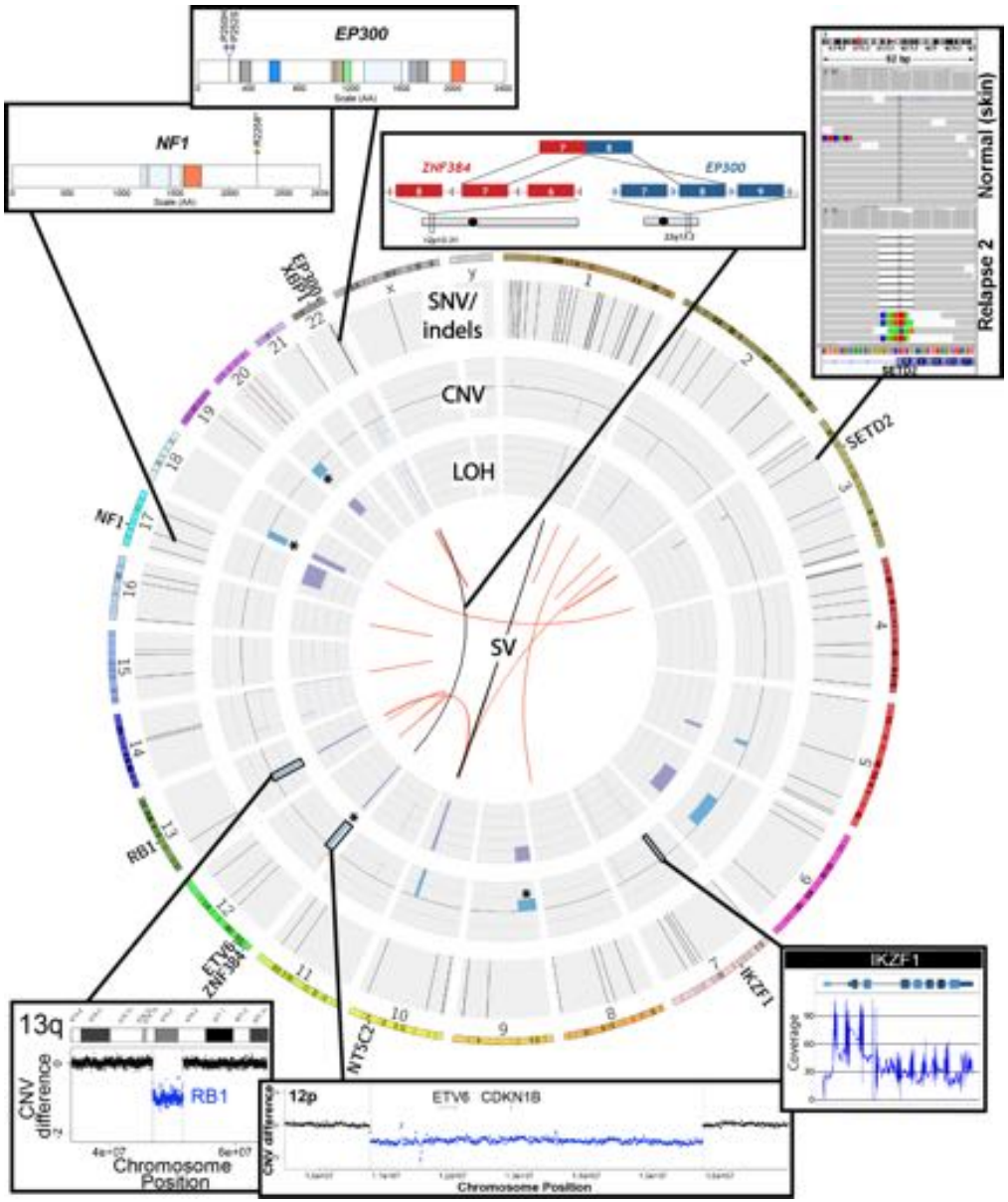
‘WGS’

‘Exome’

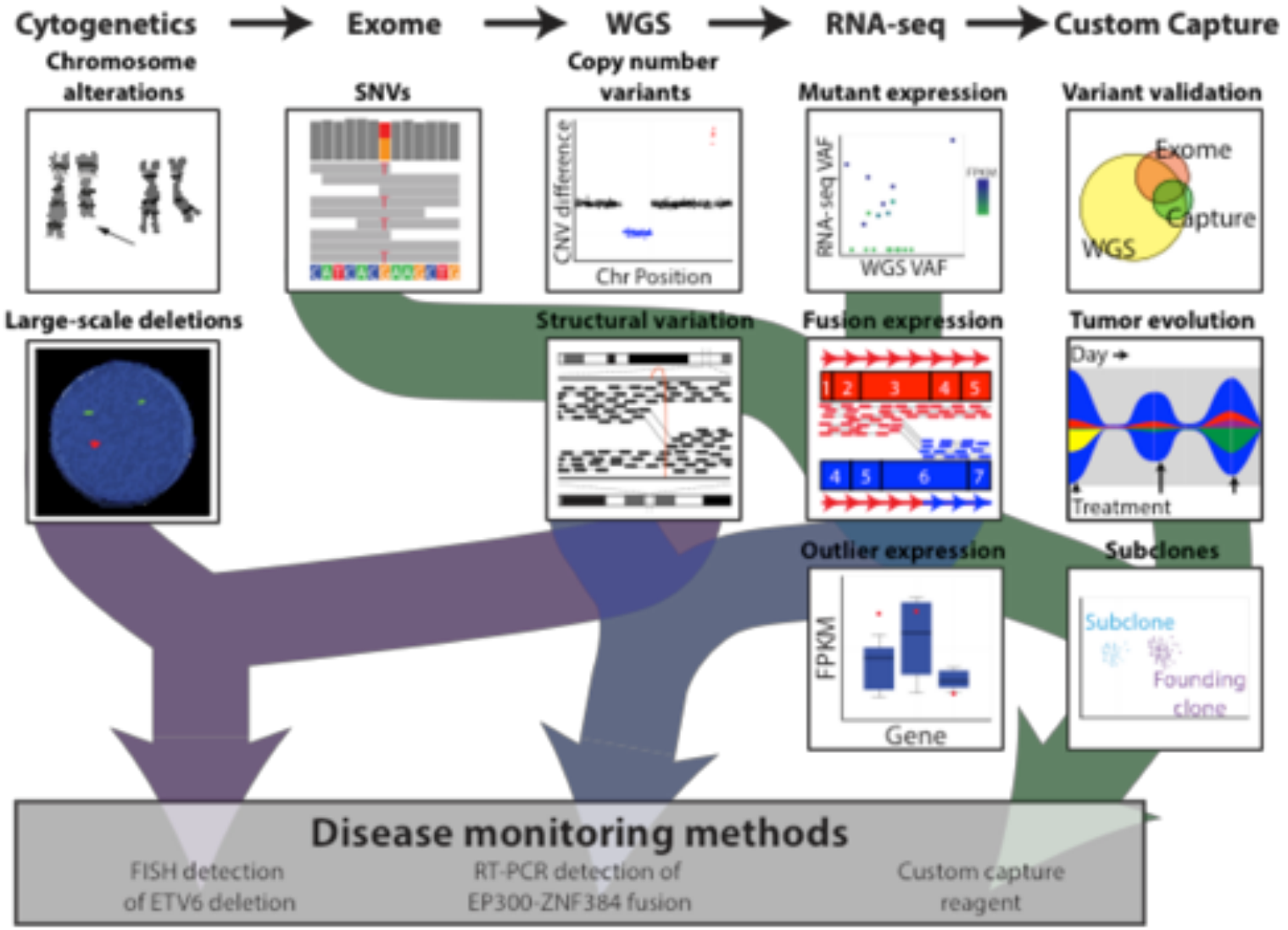
‘RNA-seq’



SNVs, Indels, CNVs, SVs, fusions, LOH, expression changes, methylation changes, and more

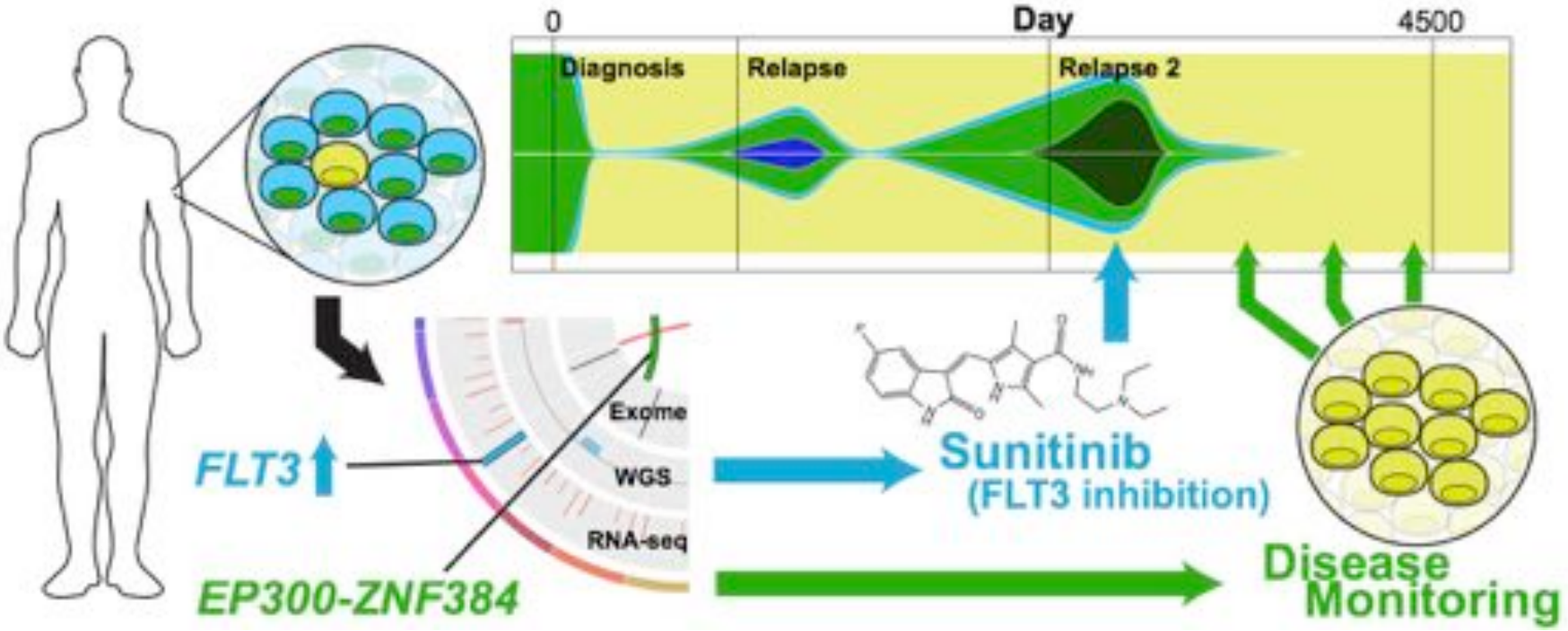


Comprehensive and integrative analysis methods are needed



Kilannin Krysiak

Personalized medicine requires personalized strategies



ELSEVIER
CrossMark
Experimental Hematology
Experimental Hematology 2016;44:603–613

Comprehensive genomic analysis reveals *FLT3* activation and a therapeutic strategy for a patient with relapsed adult B-lymphoblastic leukemia

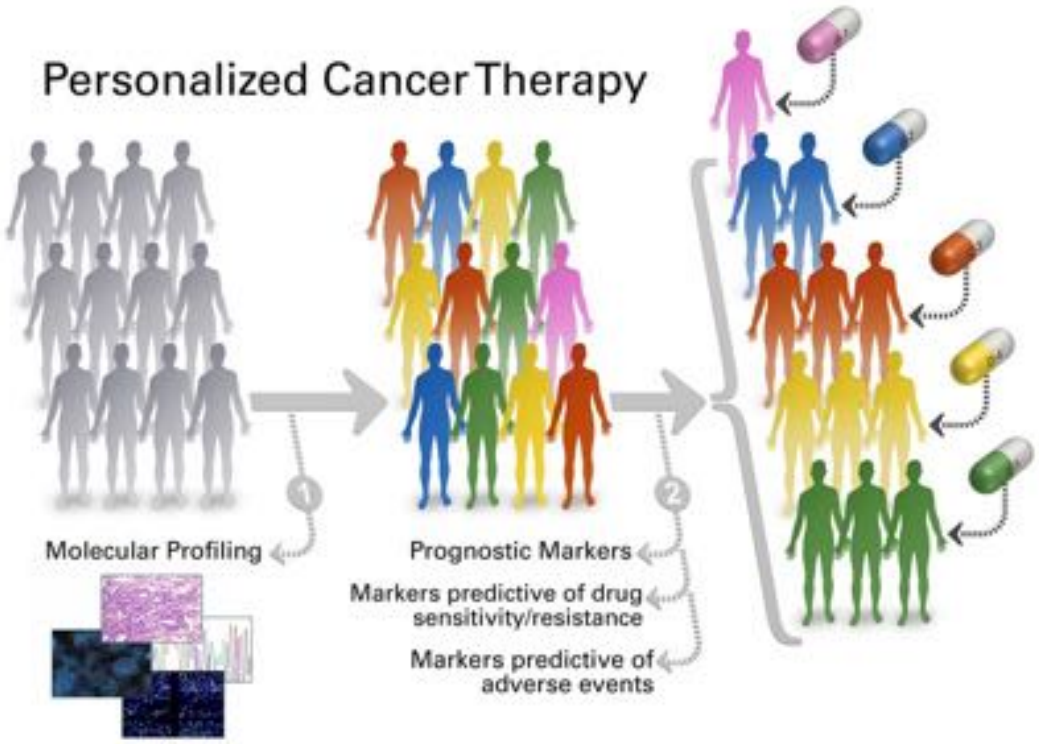
Griffith et al. 2016

Strategies to bring genomics information to bear for as many cancer patients as possible

1. **Precision medicine targeting of driver mutations**

2. Leveraging passenger variants
   a. Tracking minimal residual disease
   b. Identifying neoepitopes
      - Predicting response to immunotherapy
      - Developing personalized vaccines

Precision medicine targeting of driver mutations



1980s: Development of Targeted Therapies

2000: Human Genome Sequencing Project

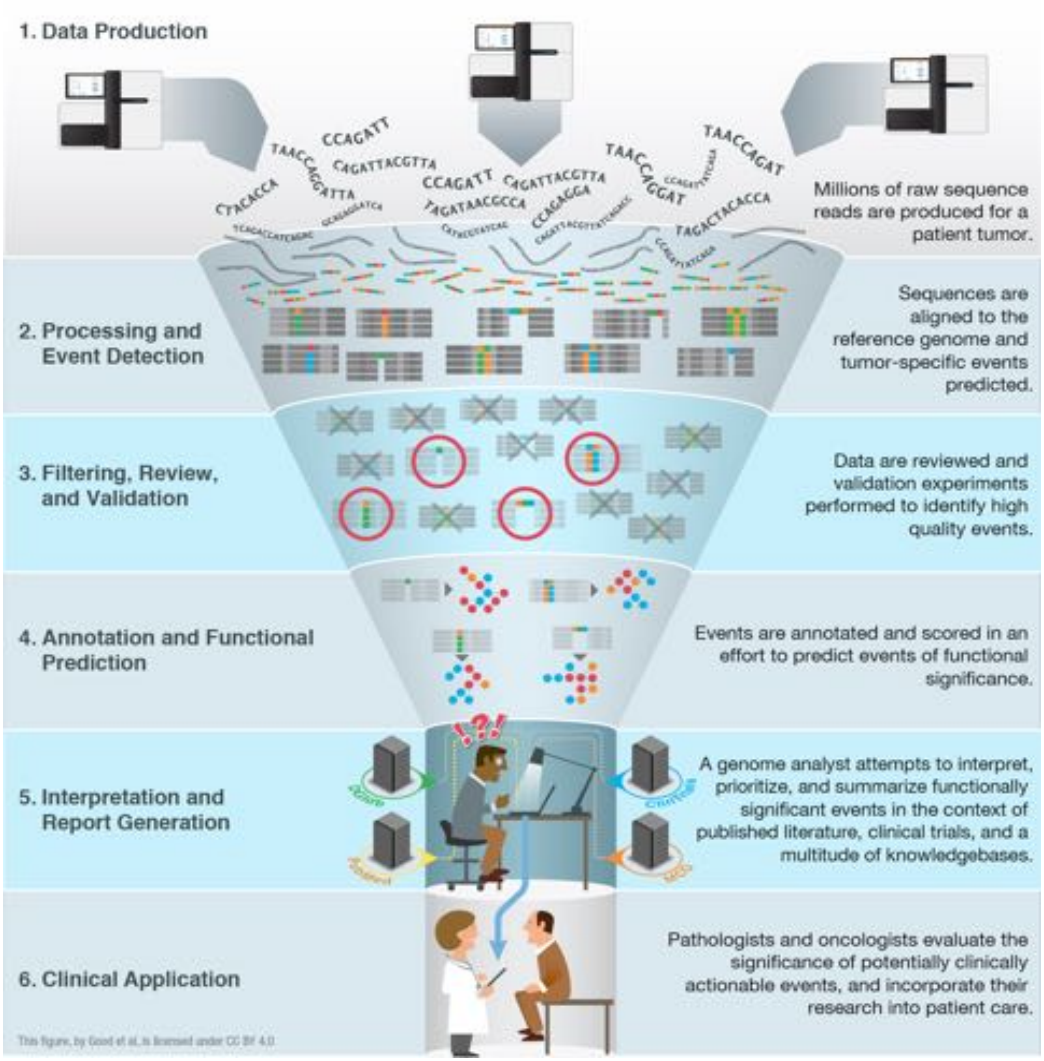
2001: FDA Approval for Imatinib in BCR-ABL1 CML

2014: FDA Approval for BRCA Testing

2017: FDA Approval of Pembro for MSI tumors

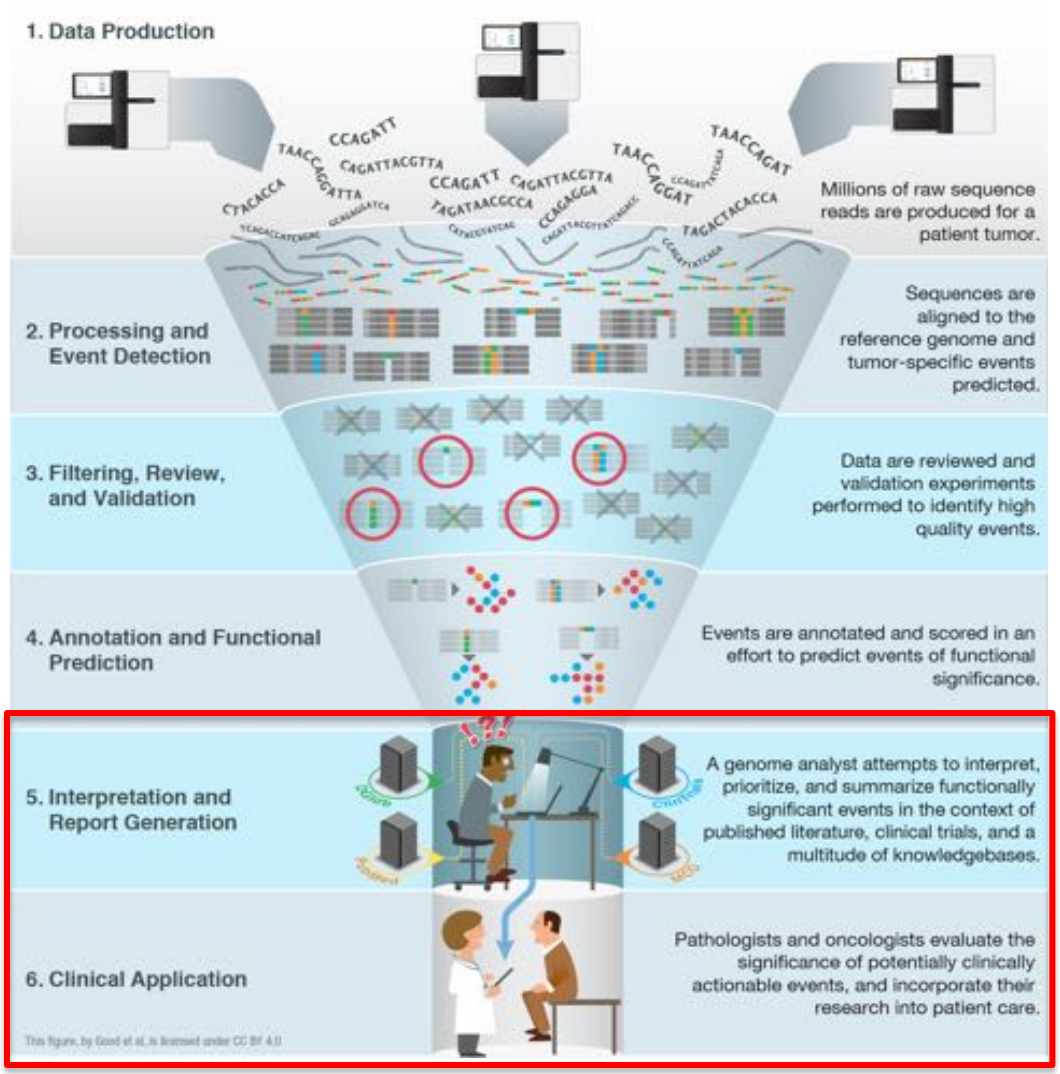
BRAF -> V600E -> Melanoma -> Predictive -> Vemurafenib
ERBB2 -> Amplification -> Breast -> Predictive -> Traztuzumab
EGFR -> L858R -> Lung -> Predictive -> Erlotinib
ALK -> Fusions -> Lung -> Predictive -> Crizotinib
EWSR1-FLI1 -> Fusions -> Ewing Sarcoma -> Diagnostic
DNAJB1-PRKACA -> Fusions -> flHCC -> Diagnostic
VHL -> Loss of function mutations -> Kidney -> Predisposing
… an increasingly long tail of rare but clinically relevant variants

High-throughput sequencing has been largely automated allowing rapid identification of somatic and germline variants



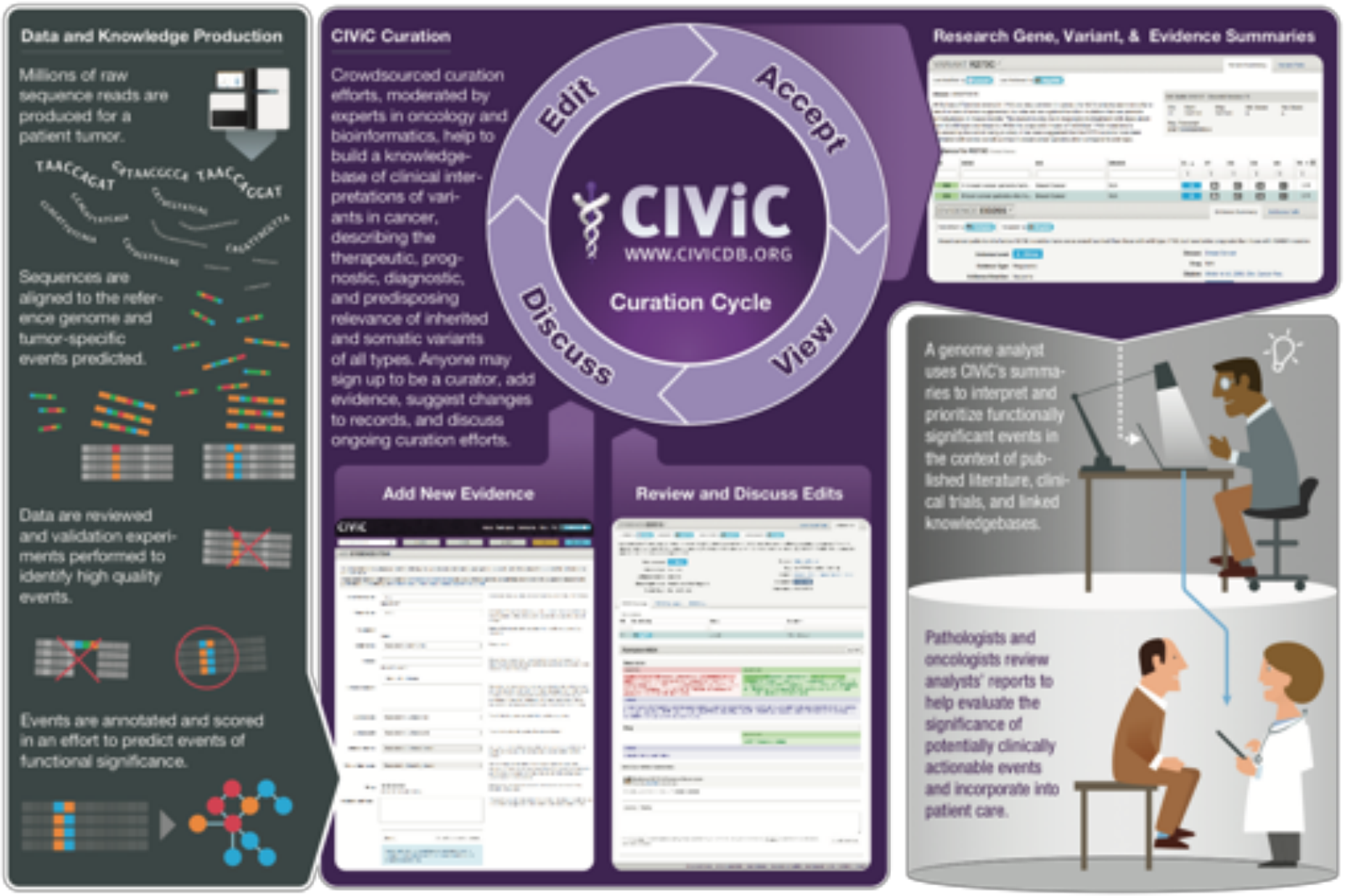
Good BM, Ainscough BJ, McMichael JF, Su AI, Griffith OL. 2014. Genome Biology. 15(8):438.

Interpretation and visualization of genomic alterations remains the bottleneck for realizing precision medicine



Good BM, Ainscough BJ, McMichael JF, Su AI, Griffith OL. 2014. Genome Biology. 15(8):438.

We created CIViC to address this need - an open knowledgebase and curation system for clinical interpretation of variants in cancer



www.civicdb.org

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   a. **Tracking minimal residual disease**
   b. Identifying neoepitopes
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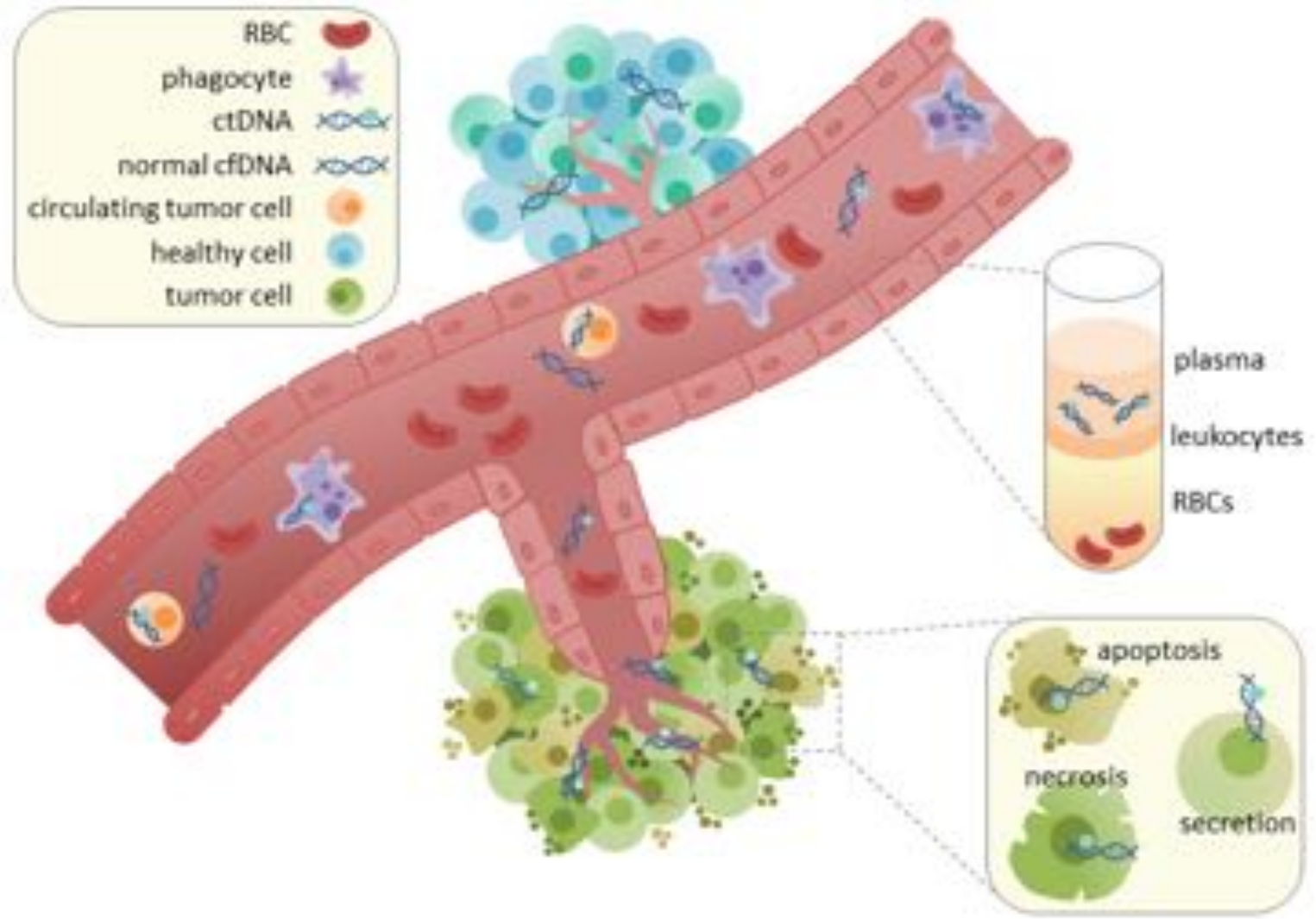
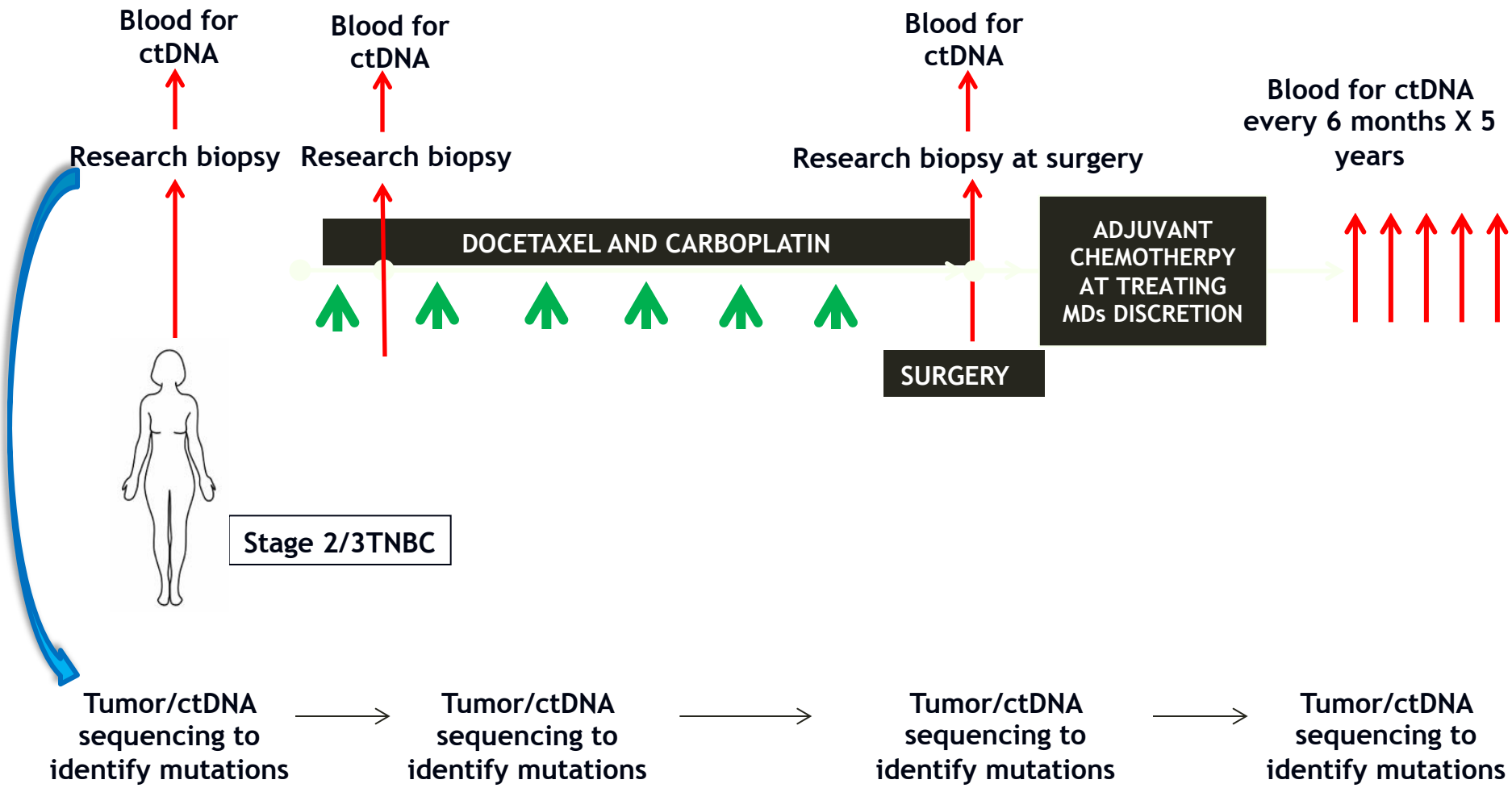
Circulating tumor DNA (ctDNA) could allow generalized tracking in any cancer type



ctDNA tracking in triple negative breast cancer



Clinical Trial: NCT02124902 (Contact PI: Foluso Ademuyiwa)

Strategies to bring genomics information to bear for as many cancer patients as possible

1. Precision medicine targeting of driver mutations

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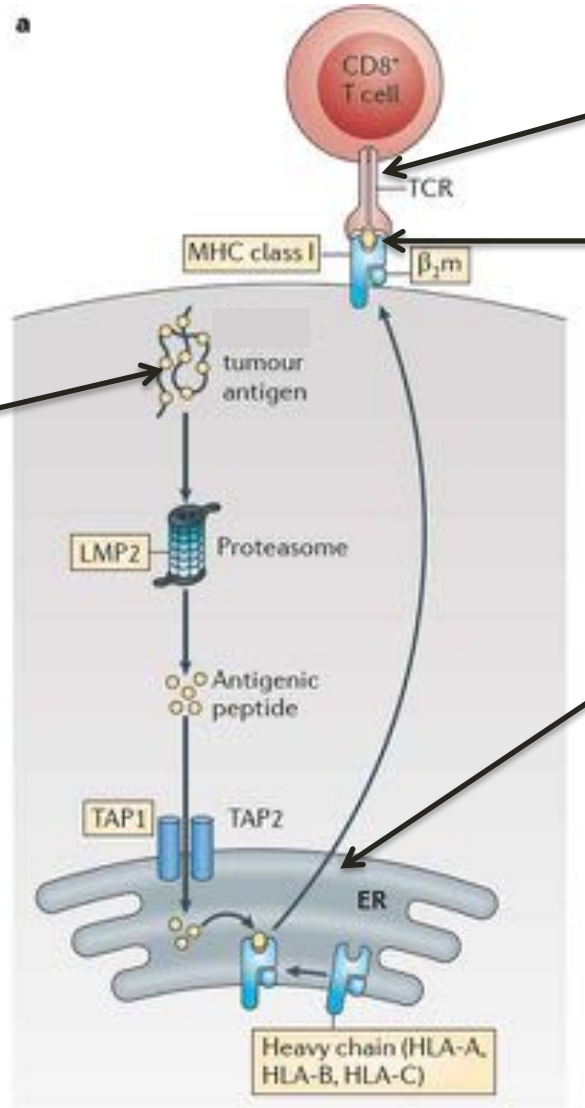
   a. Tracking minimal residual disease
   b. **Identifying neoepitopes**
      - **Predicting response to immunotherapy**
      - **Developing personalized vaccines**

Invoking an adaptive immune response against the tumor (focus on CD8+ T cells)

1. Tumor produces a unique peptide corresponding to a somatic **mutation**

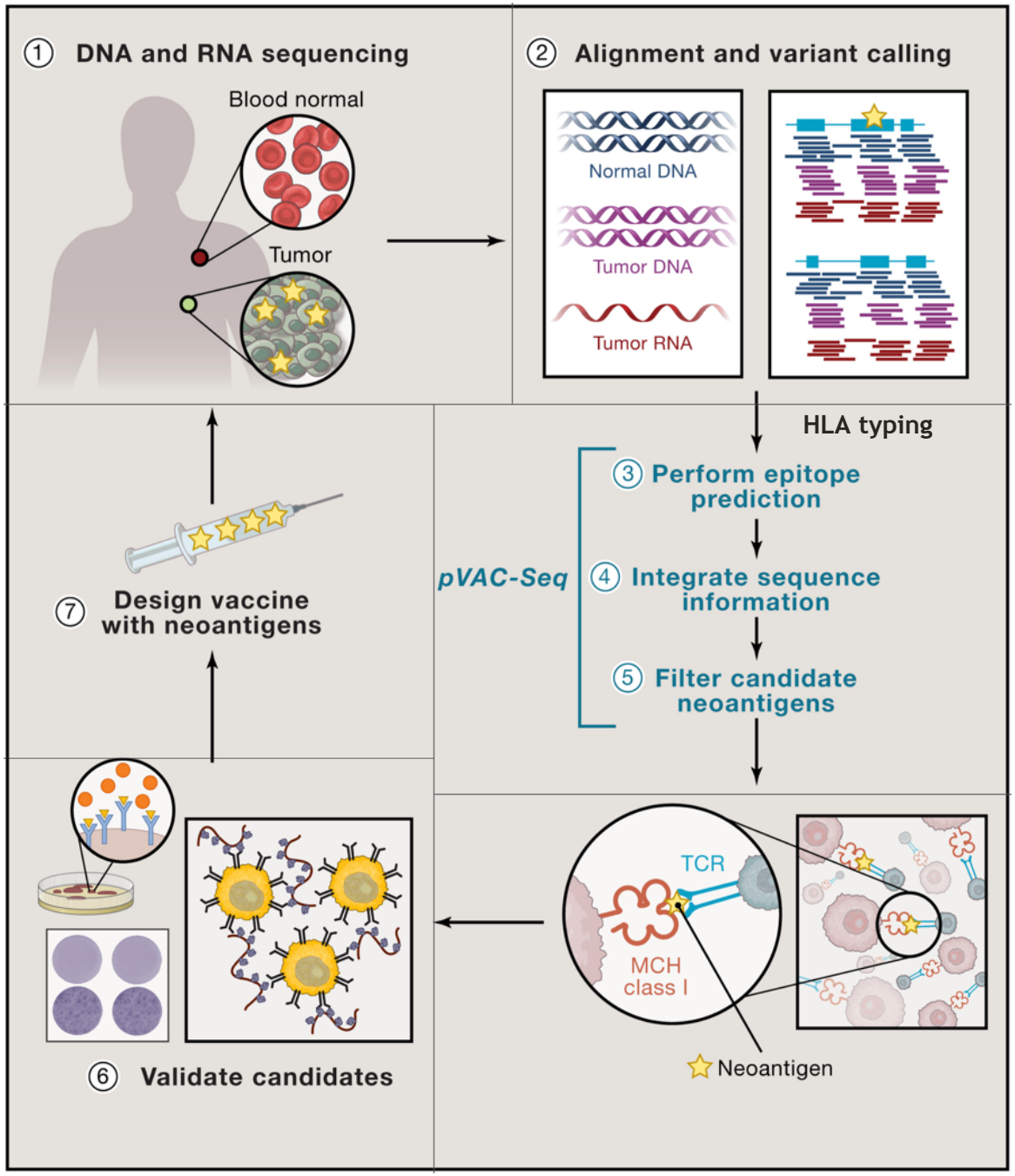


4. T cell receptor that uniquely matches the tumor peptide

3. Neoantigen peptide presented by **MHC**

2. Processing and presentation of the tumor specific peptide

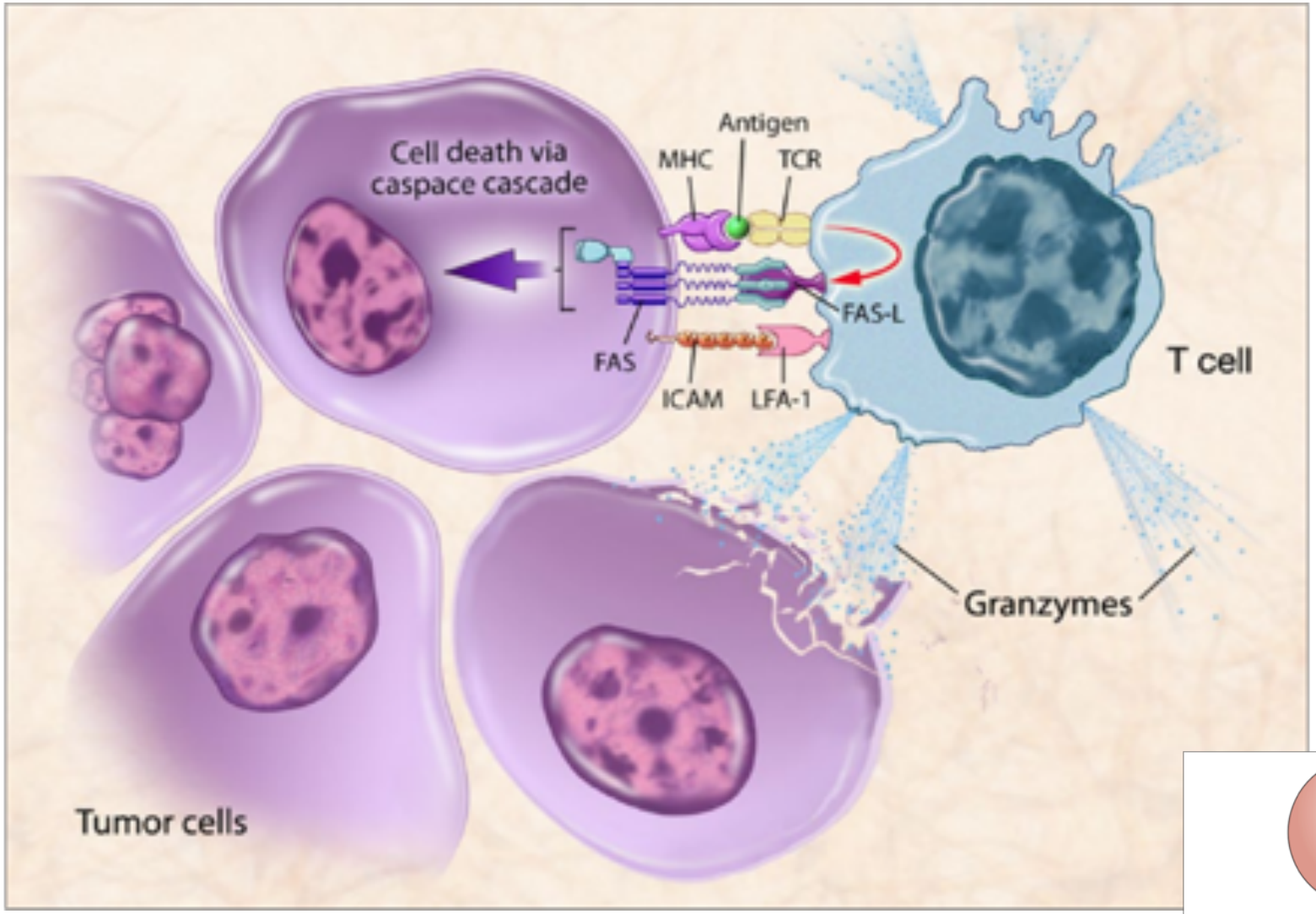
Neoepitope characterization workflow

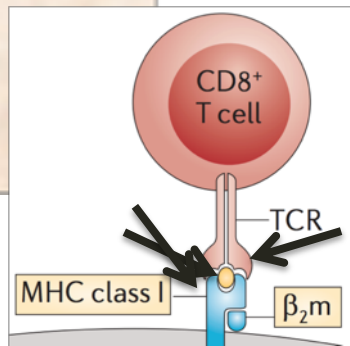


Jasreet Hundal & Katie Campbell

Shirley X. Liu, Elaine R. Mardis. Applications of Immunogenomics to Cancer. Cell Press. 2017.

T cell mediated cell death





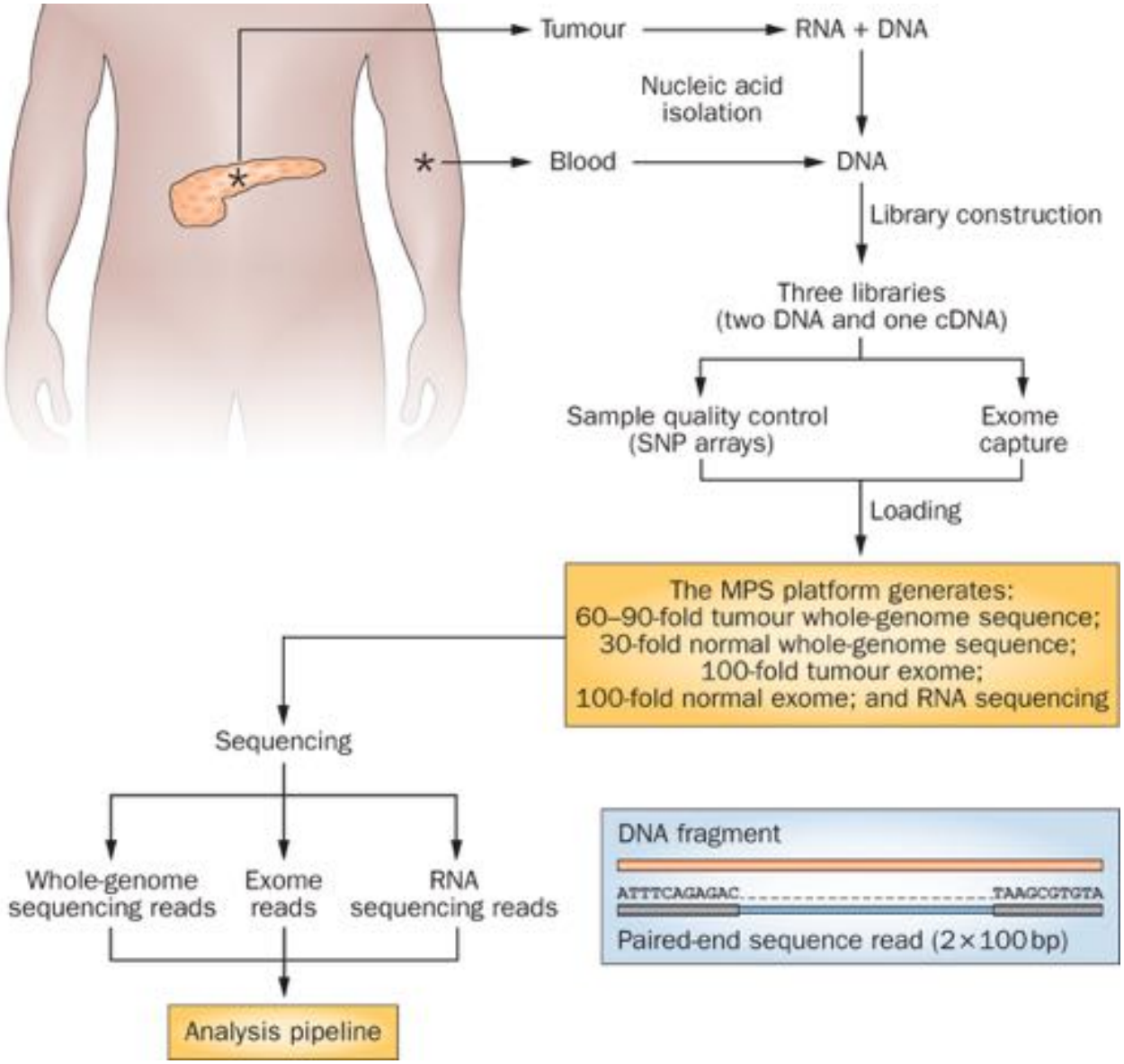
Singh and Gulley, 2014

Personalized cancer vaccine trials

- Kidney Cancer (n = 15 patients)
  - PolyImmune {Durvalumab (MEDI4736) and Tremelimumab} & Vaccine Orchestrated Treatment for Patients With Advanced/Metastatic Renal Cell Carcinoma (NCT03598816). Collaboration with MedImmune.
- Lung Cancer (n = 15 patients)
  - A Personal Cancer Vaccine (NEO-PV-01) With Pembrolizumab and Chemotherapy for Patients With Lung Cancer (NCT03380871). Collaboration with Neon Therapeutics and Merck.
- Glioblastoma (n = 30 patients)
  - Neoantigen-based Personalized Vaccine Combined With Immune Checkpoint Blockade Therapy in Patients With Newly Diagnosed, Unmethylated Glioblastoma (NCT03422094)
- Breast Cancer (n = 54 patients)
  - Neoantigen DNA Vaccine Alone vs. Neoantigen DNA Vaccine Plus Durvalumab in Triple Negative Breast Cancer Patients Following Standard of Care Therapy (NCT03199040). Collaboration with MedImmune.
  - Safety and Immunogenicity of a Personalized Polyepitope DNA Vaccine Strategy in Breast Cancer Patients With Persistent Triple-Negative Disease Following Neoadjuvant Chemotherapy (NCT02348320)
- Follicular Lymphoma (n = 20 patients)
  - Personalized Tumor Vaccine Strategy and PD-1 Blockade in Patients With Follicular Lymphoma (NCT03121677). Collaboration with Bristol-Myers Squibb.
- Prostate Cancer (n = 20 patients)
  - Neoantigen DNA Vaccine in Combination With Nivolumab/Ipilimumab and PROSTVAC in Metastatic Hormone-Sensitive Prostate Cancer (NCT03532217). Collaboration with Bristol-Myers Squibb.
- Pancreatic Cancer (n = 15 patients)
  - Neoantigen DNA Vaccine in Pancreatic Cancer Patients Following Surgical Resection and Adjuvant Chemotherapy (NCT03122106).
- Melanoma (n = 12 patients)
  - Dendritic Cell Vaccination in Patients With Advanced Melanoma (NCT03092453). Collaboration with UPenn/Parker ICI.

Genomic data visualization and interpretation

Genomics research has exploded with the rapid advances in DNA sequencing technologies

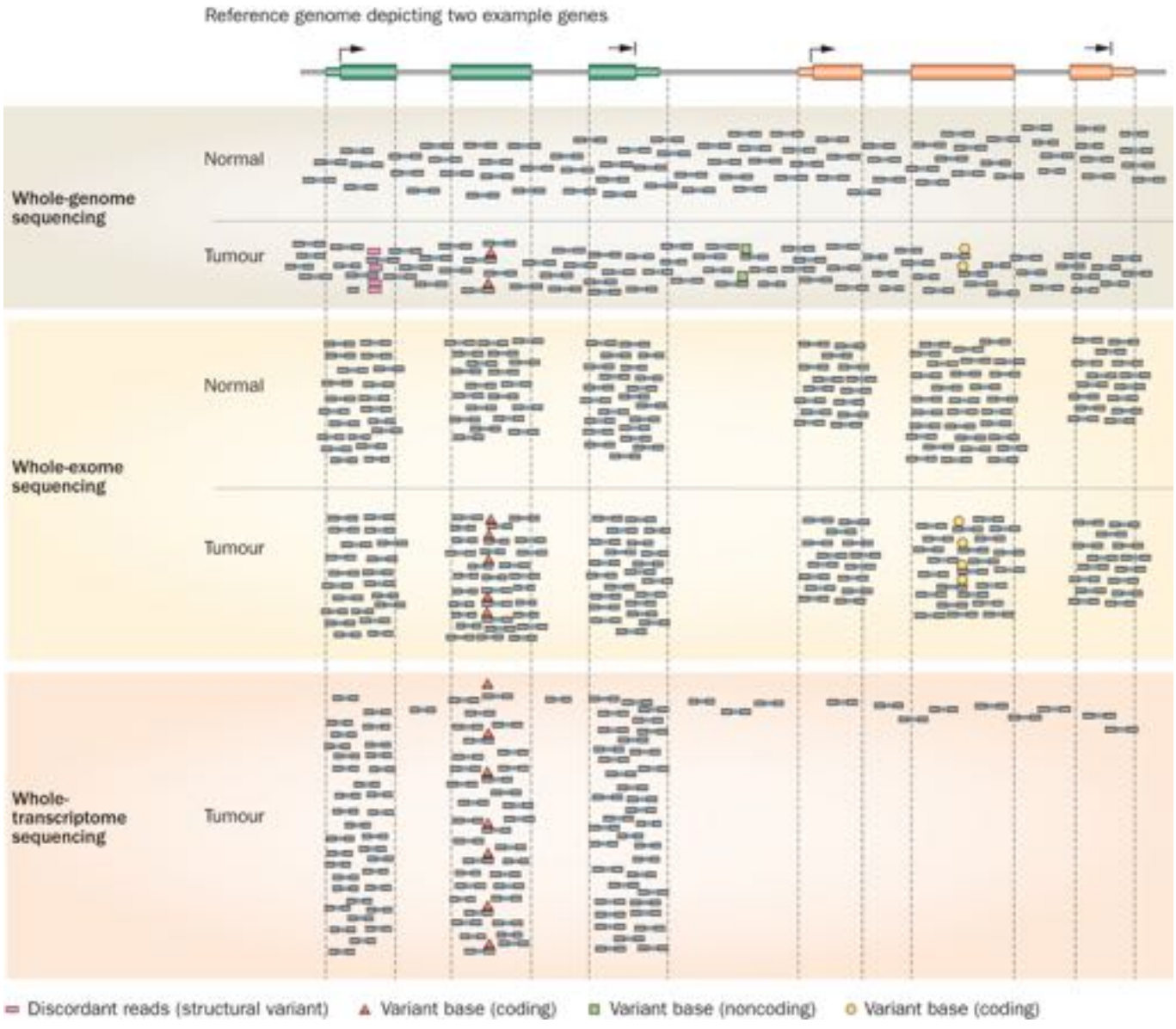


Whole genome, exome and transcriptome sequencing allows us to detect and confirm many different variant types

‘WGS’

‘Exome’

‘RNA-seq’



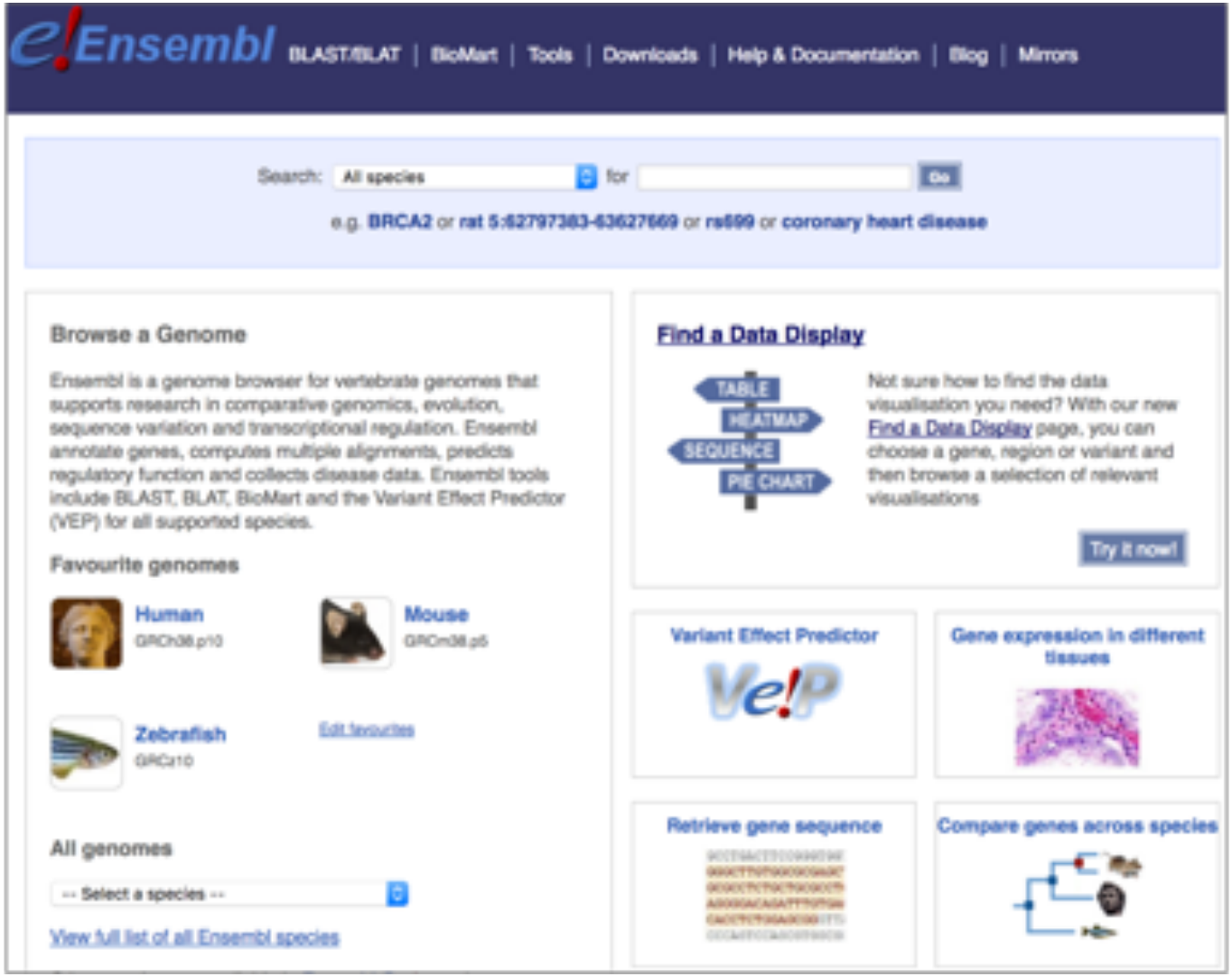
Why do we create visualizations of genomic data?

- Data exploration and interpretation of results
  - QC analysis
  - Understanding whether/how an experiment worked
  - Discovery

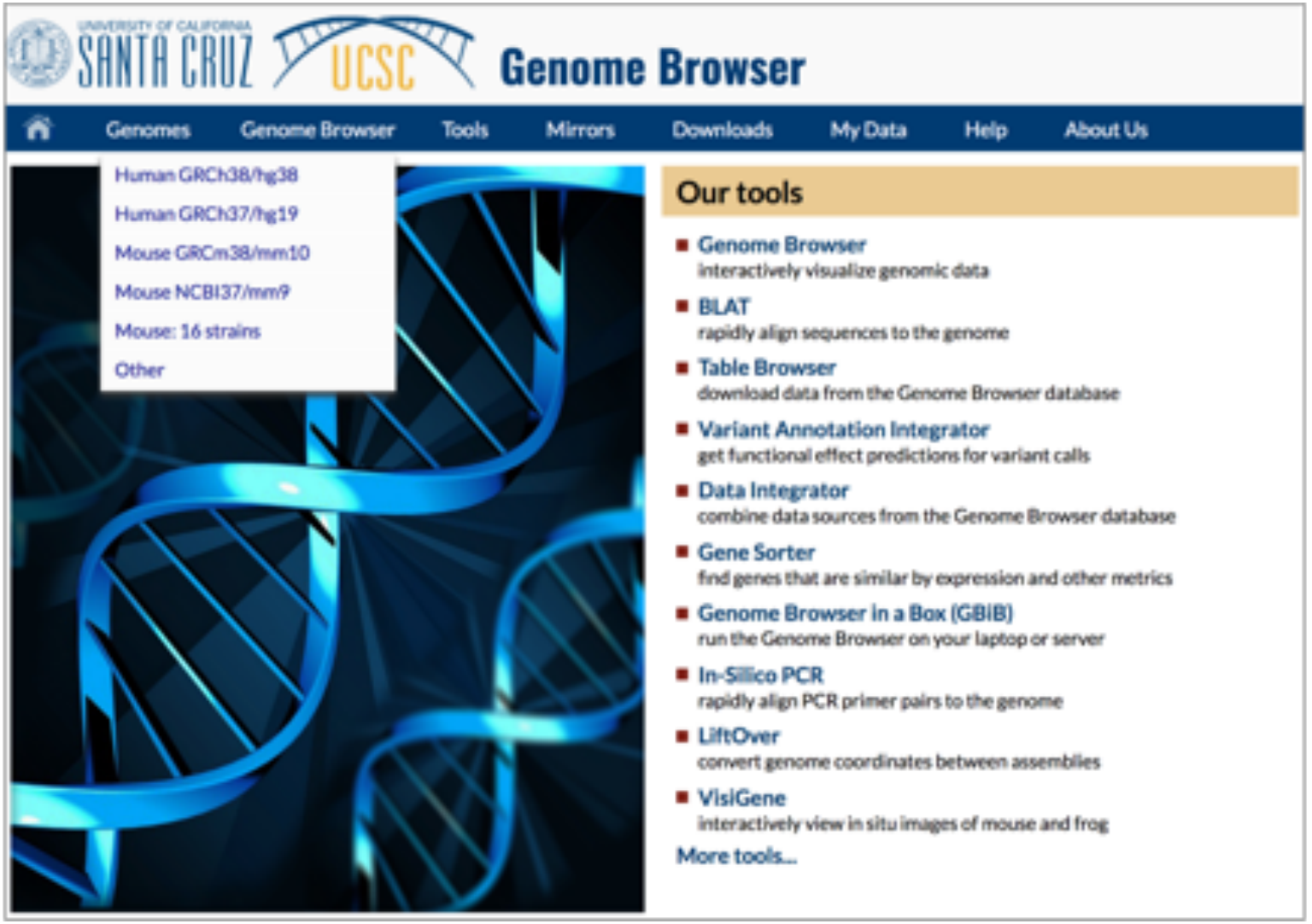
- Communication
  - Slides for presentations
    - e.g. Keynote, Powerpoint, etc.
  - Figures for publications
    - e.g. PDFs, PNGs, etc.
      - Illustrator, Gimp, Inkscape, etc.
      - R and R Studio

Genome browsers

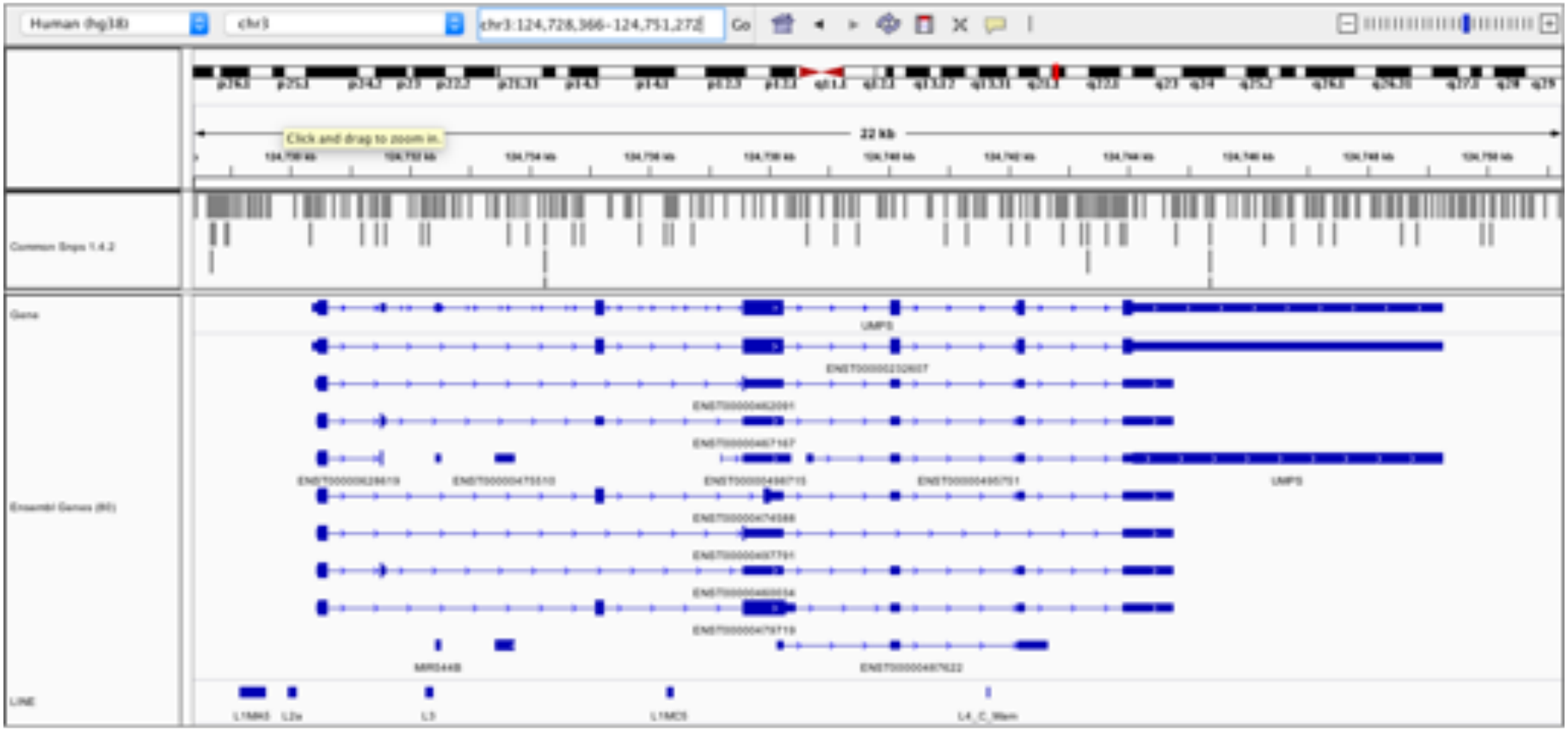
Genome browsers - Ensembl



Genome browsers - UCSC



Genome browsers - IGV

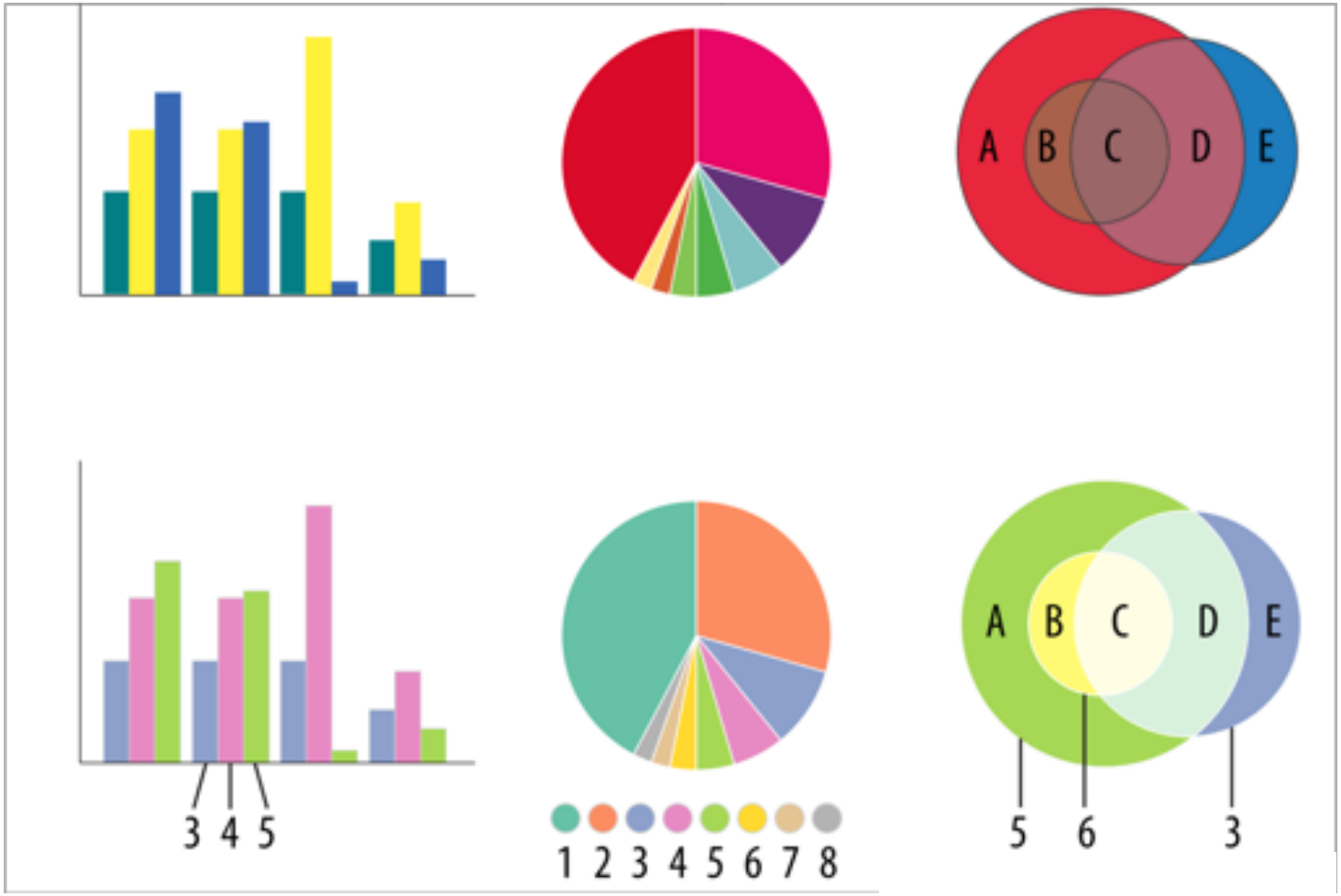


Fundamentals of data visualization

Fundamentals of data visualization

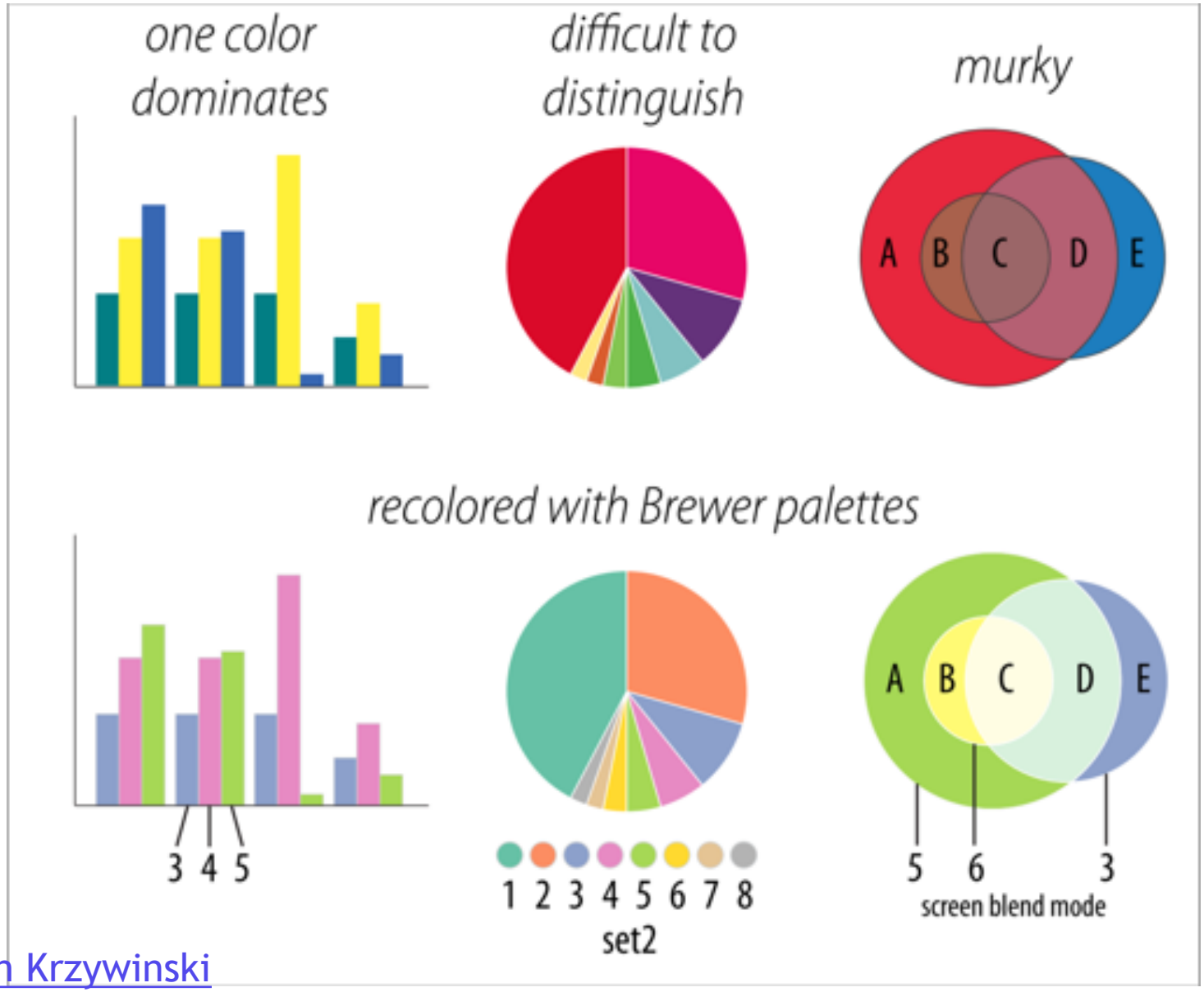
- Where to learn more about the art and science of visualization:
  - Collection of 40 Nature Methods articles on data visualization: "Points of View" Articles
  - Visual design principles lecture

Which series is more effective (top or bottom)?



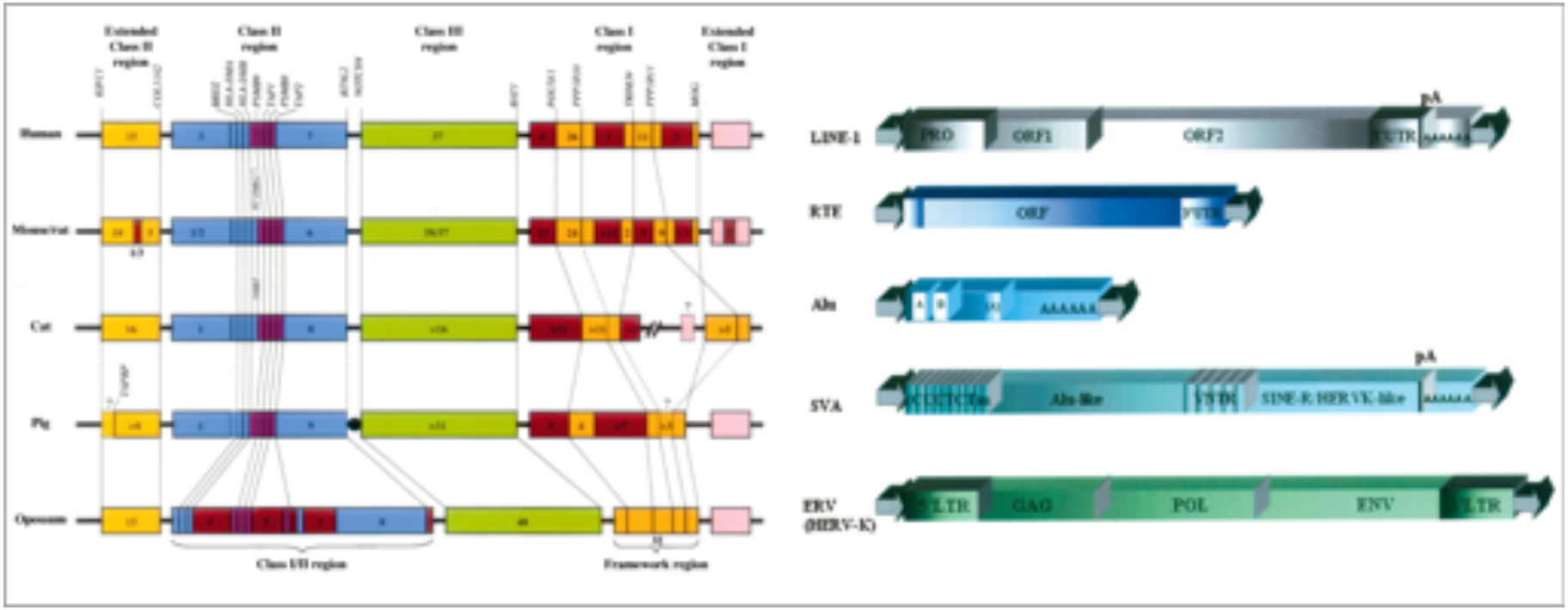
Martin Krzywinski

Which series is more effective (top or bottom)?



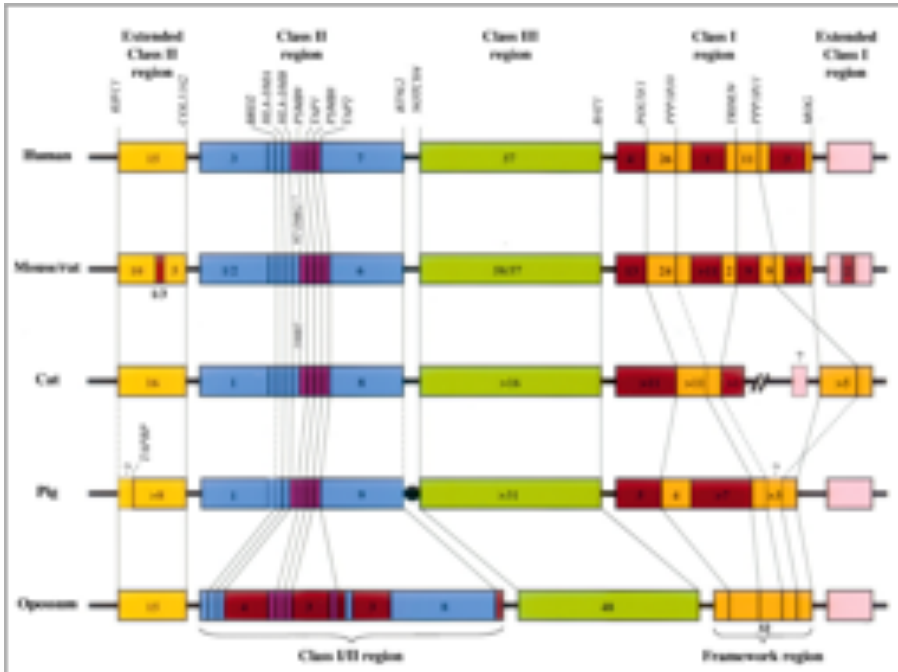
Martin Krzywinski

Which is more effective (left or right?)



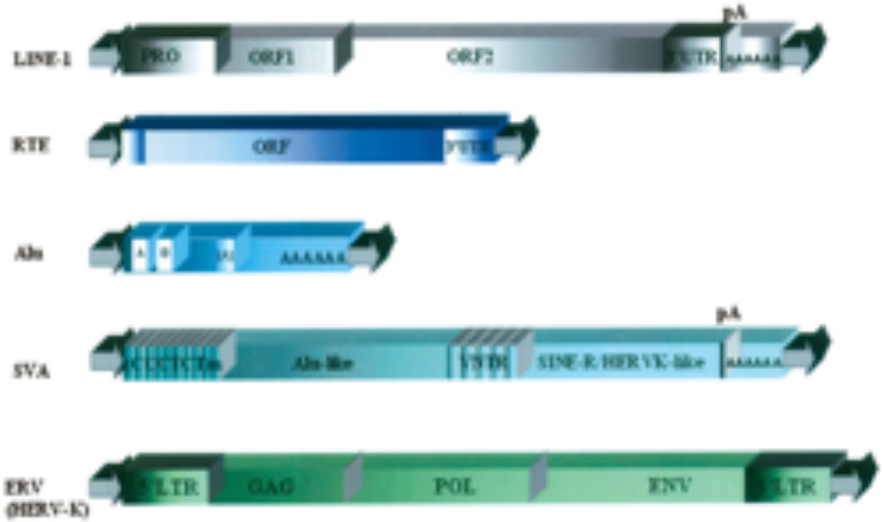
Martin Krzywinski

Which is more effective (left or right?)



Excellent organization and consistency. Vertical lines cue continuity. Good use of color.

Sarmollow, P.B., The opossum genome: insights and opportunities from an alternative mammal. Genome Res, 2008. 18(8): p. 1199-215.

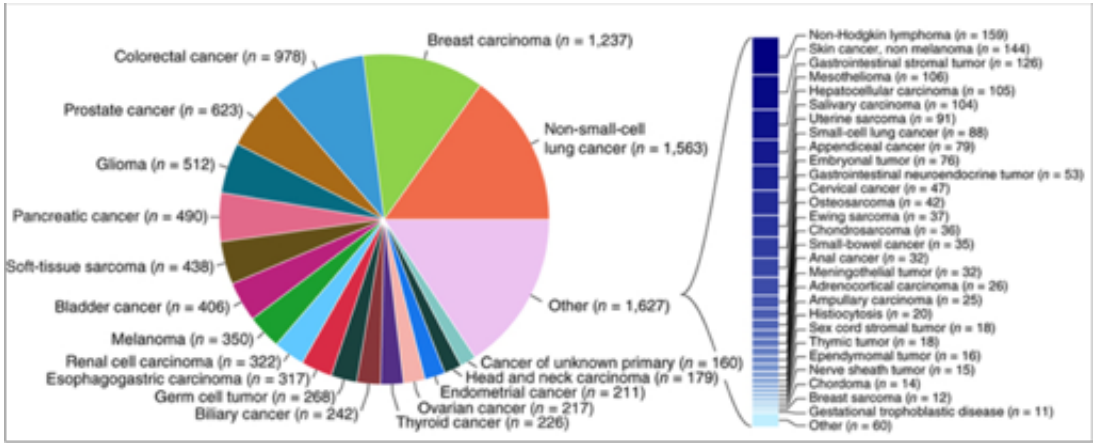


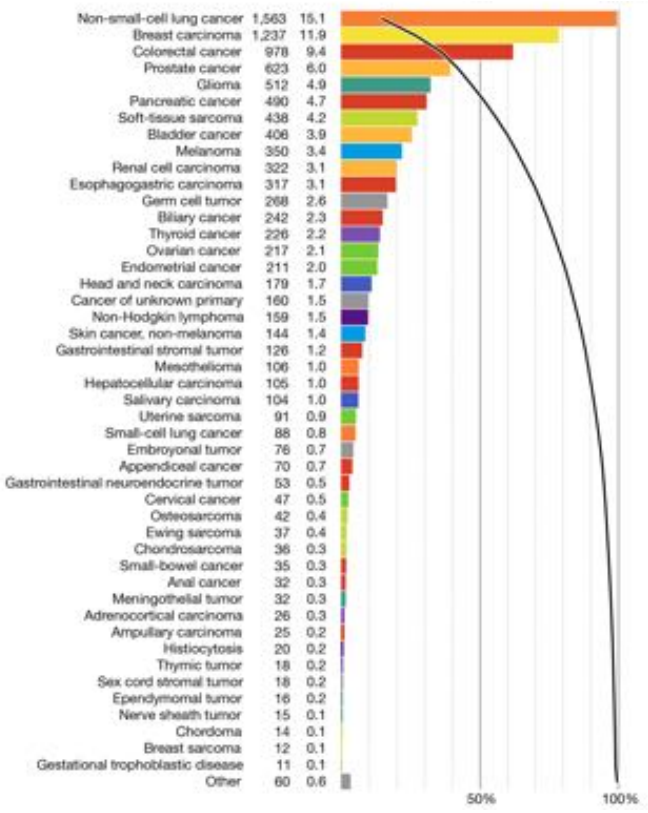
Chartjunk plentiful. Screaming ornamental and redundant elements. Text inconsistent and illegible.

Genties, A.J., et al., Evolutionary dynamics of transposable elements in the short-tailed opossum Monodelphis domestica. Genome Res, 2007. 17(7): p. 992-1004.

Martin Krzywinski

Which is more effective (left or right?)





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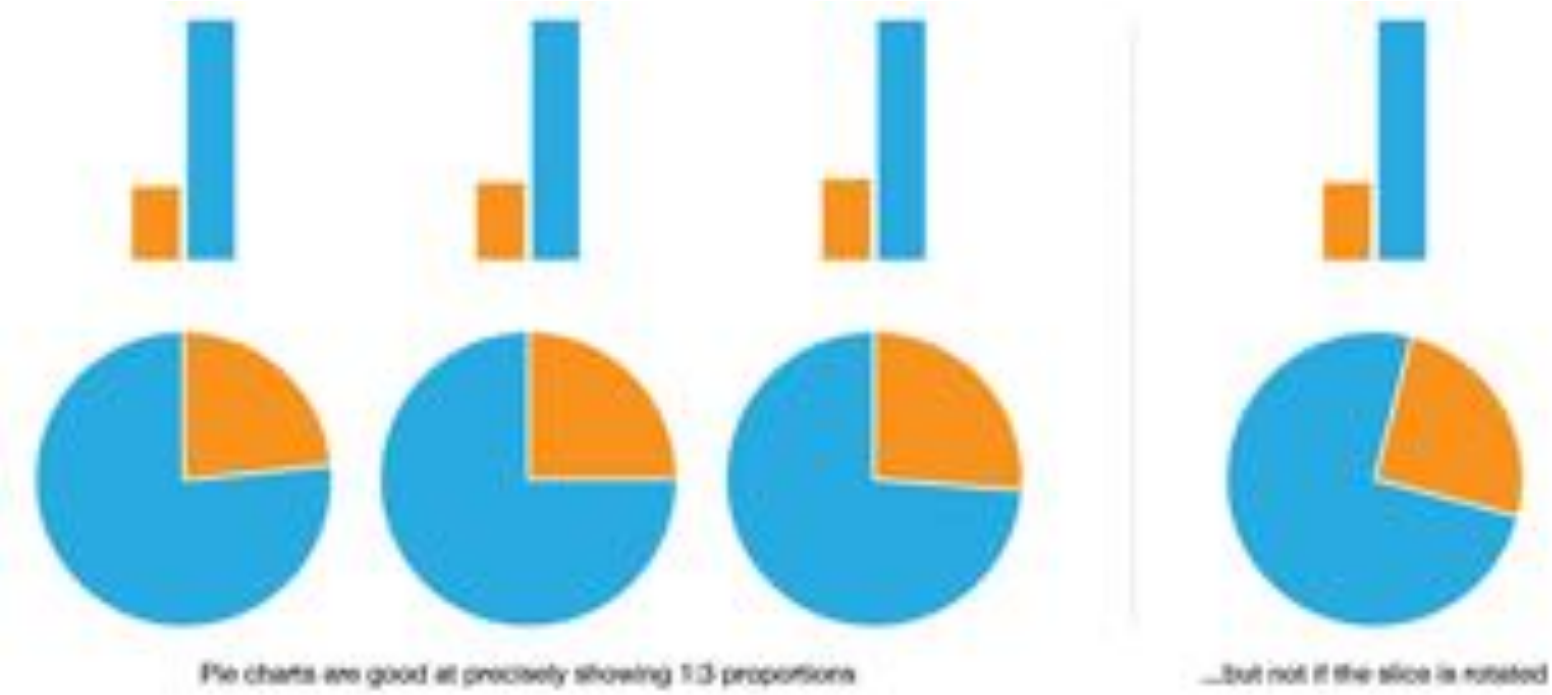
When to use a pie chart



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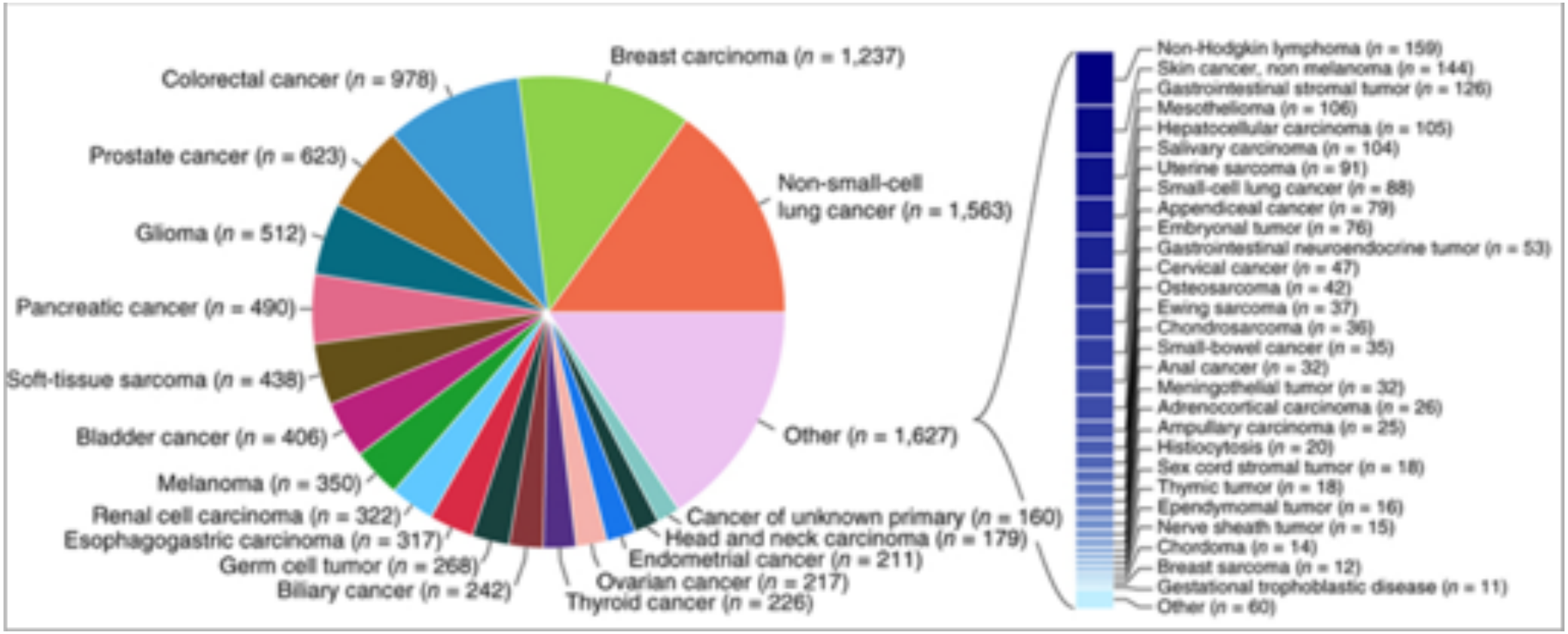
When to not use a pie chart

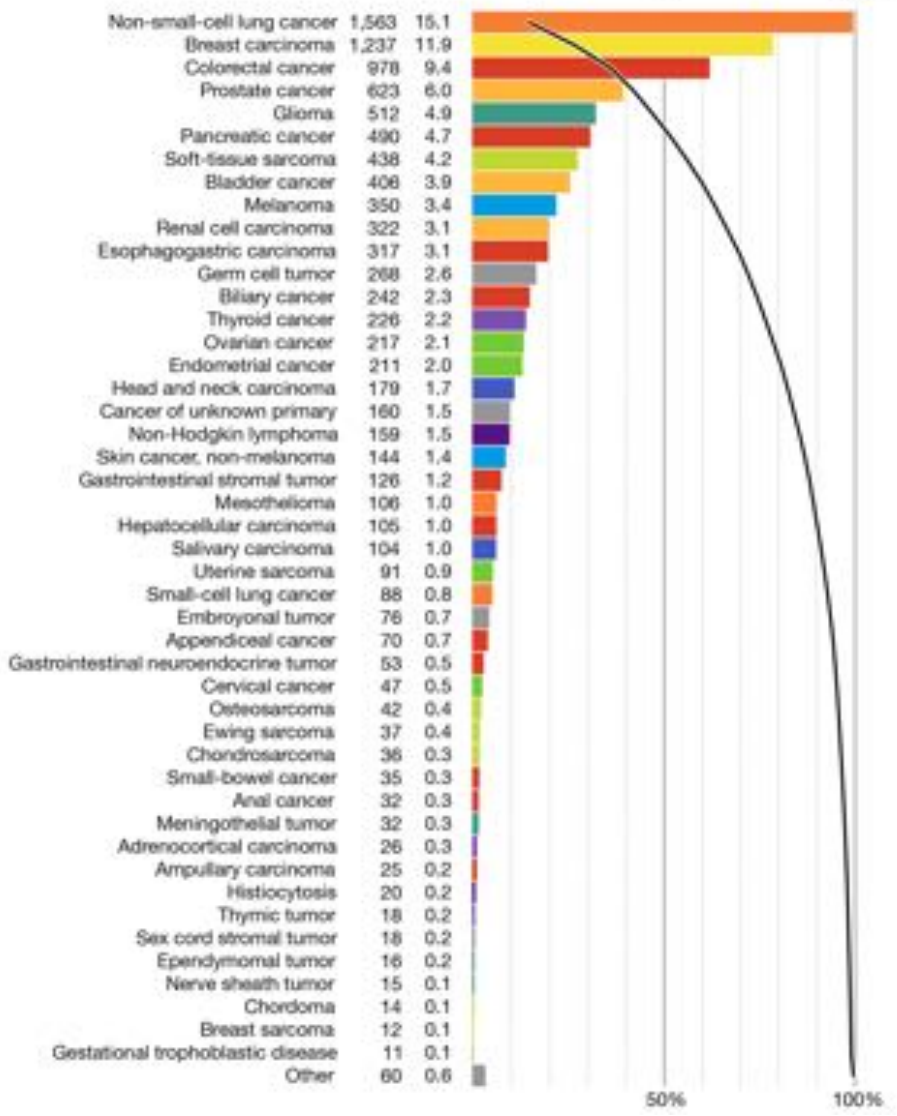


Ahmet Zehir et al. (2017) Nature Medicine doi:10.1038/nm.4333

- Hard to judge proportions
- Poor use of color
- Hard to read labels
- Over ½ of the categories had to broken out of the pie chart

Martin Krzywinski

Same data with a redesigned approach



Martin Krzywinski

Selected articles on fundamentals of data viz

- Visualizing samples with box plots
- Circos plots
- When to use (and not use) pie charts

- Resources for choosing colors
  - http://colorbrewer2.org/
  - http://mkweb.bcgsc.ca/color/
  - Understanding and using Color Palettes
  - Color palettes for color blindness
  - Names for >9000 colors
    - Including 40 beer colors

- Credit to Martin Krzywinski for his extensive work in this area and many of the above resources

Best practices in visualization

Best practices from this workshop

- *Always* label axes
- Consider readability of font size
- Avoid vertical or angled text if possible
- Avoid unnecessary use of color, point shapes, etc.
- Chose colors wisely
- If individual data points are being plotted and have started to really pile up on top of each other consider using a density function
- Always be transparent about what data manipulation is taking place (e.g. log scale, filtering of outliers, etc.)

Best practices from the experts

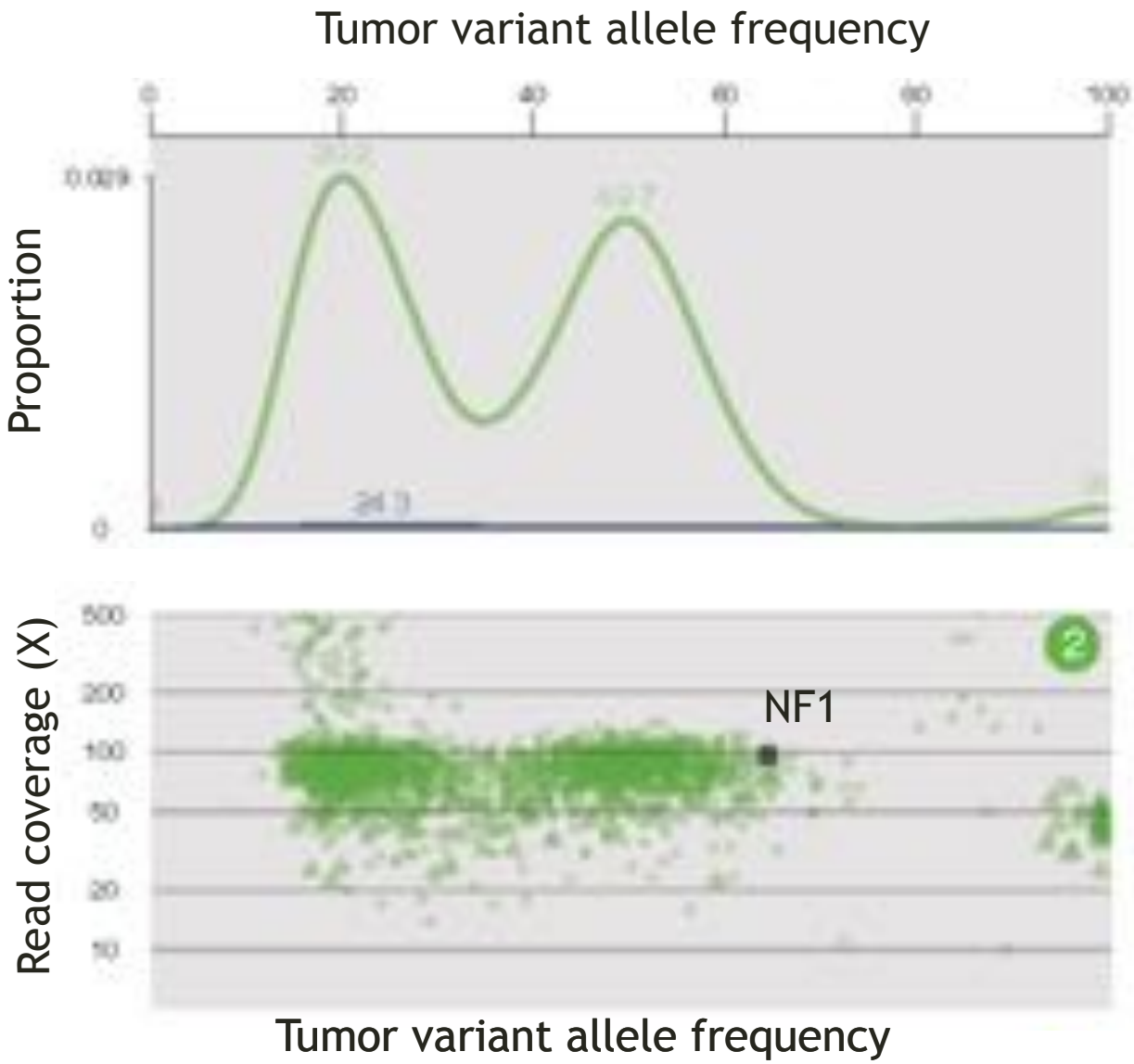
Ten Simple Rules for Better Figures (Rougier et al. 2014):

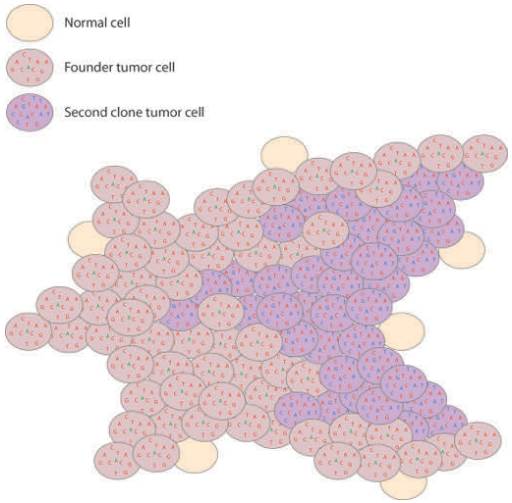
Scientific visualizations should act as a “a graphical interface between people and data”. Try to follow these rules.

1. Know Your Audience
2. Identify Your Message
3. Adapt the Figure to the Support Medium
4. Captions Are Not Optional
5. Do Not Trust the Defaults
6. Use Color Effectively
7. Do Not Mislead the Reader
8. Avoid “Chartjunk”
9. Message Trumps Beauty
10. Get the Right Tool

Example visualizations using R

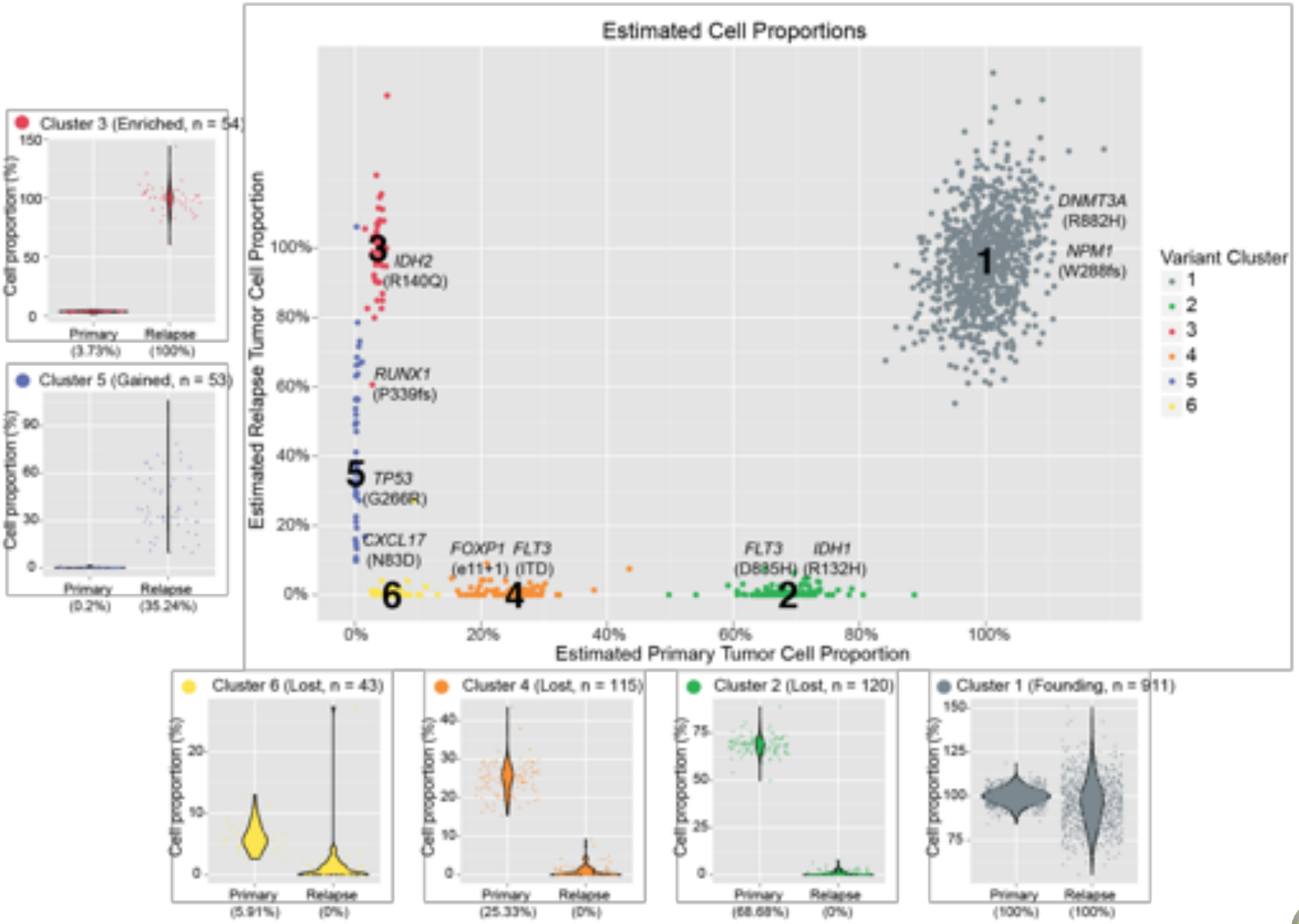
Histogram and scatterplot to define cell populations



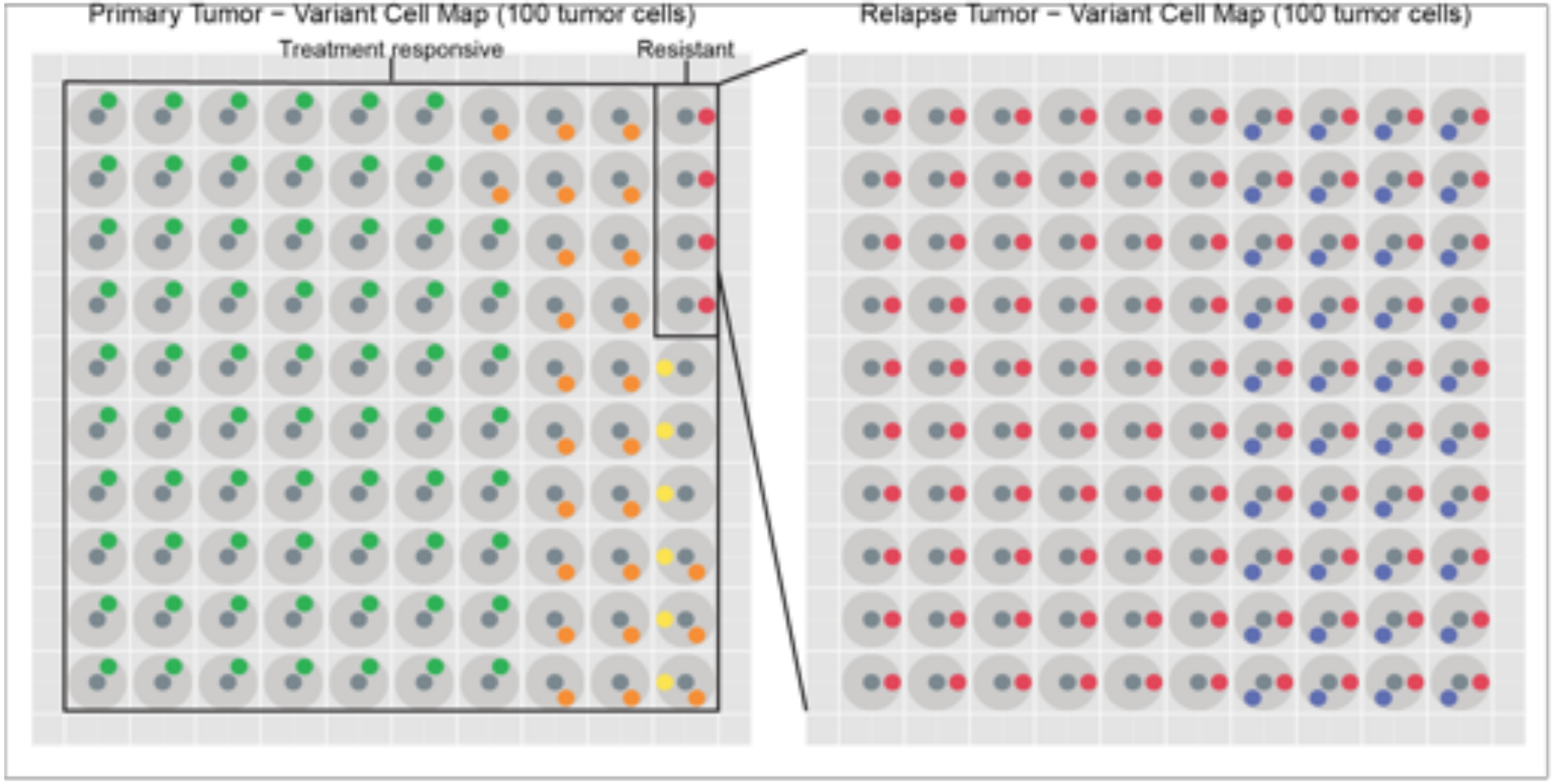


WGS data

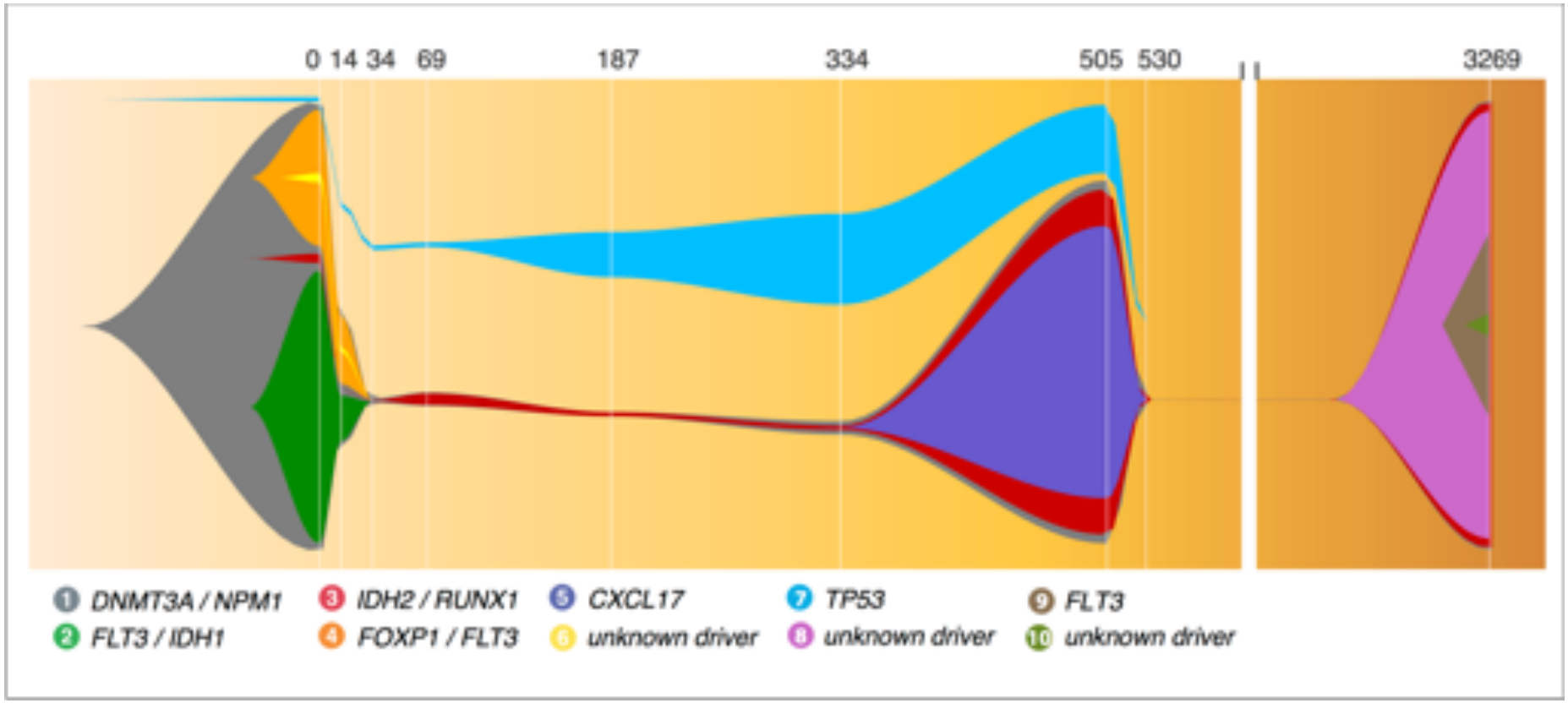
Clustering to define to distinct cell populations



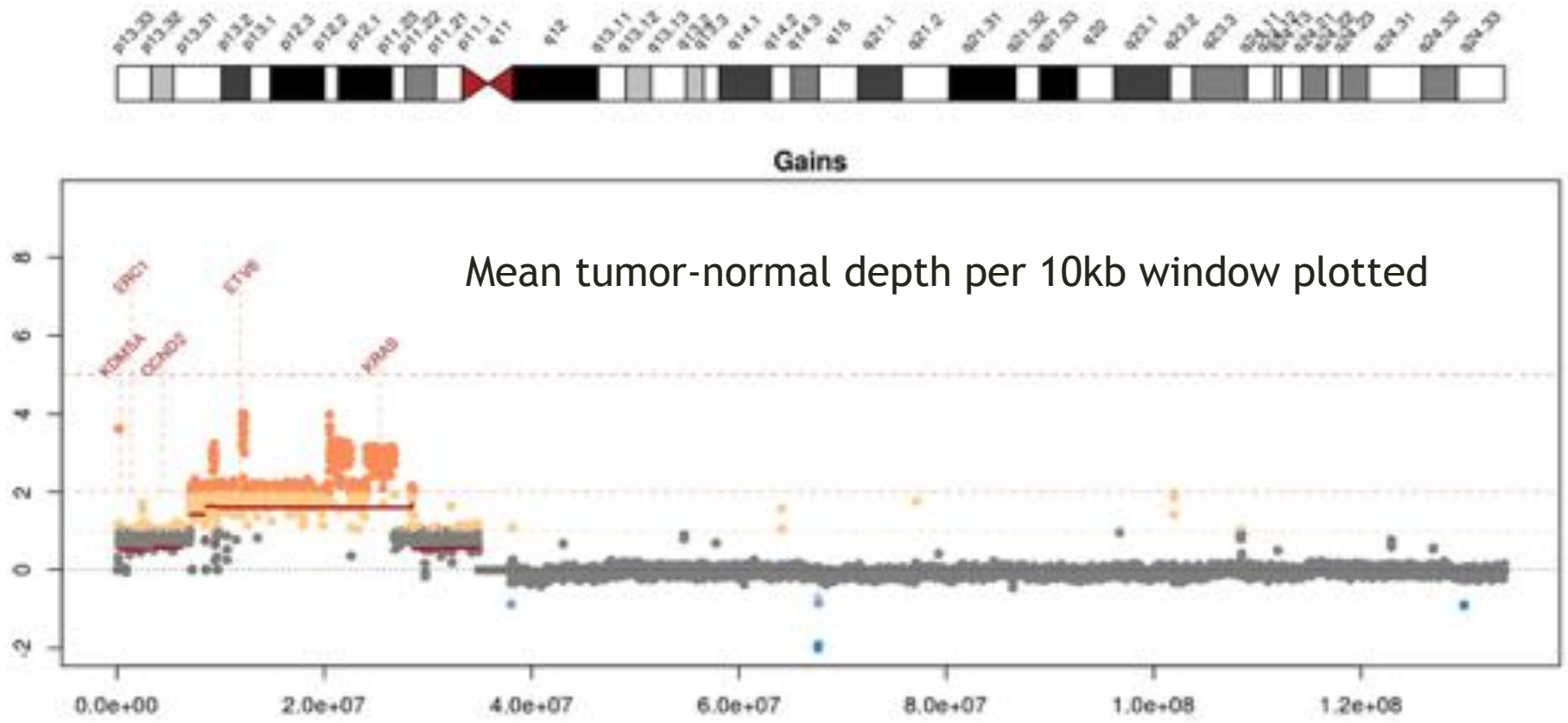
Using a “cell map” to represent the variant clusters in 100 hypothetical cells of a tissue



A ‘fish’ plot is used to represent sub-clones lost and gained over time

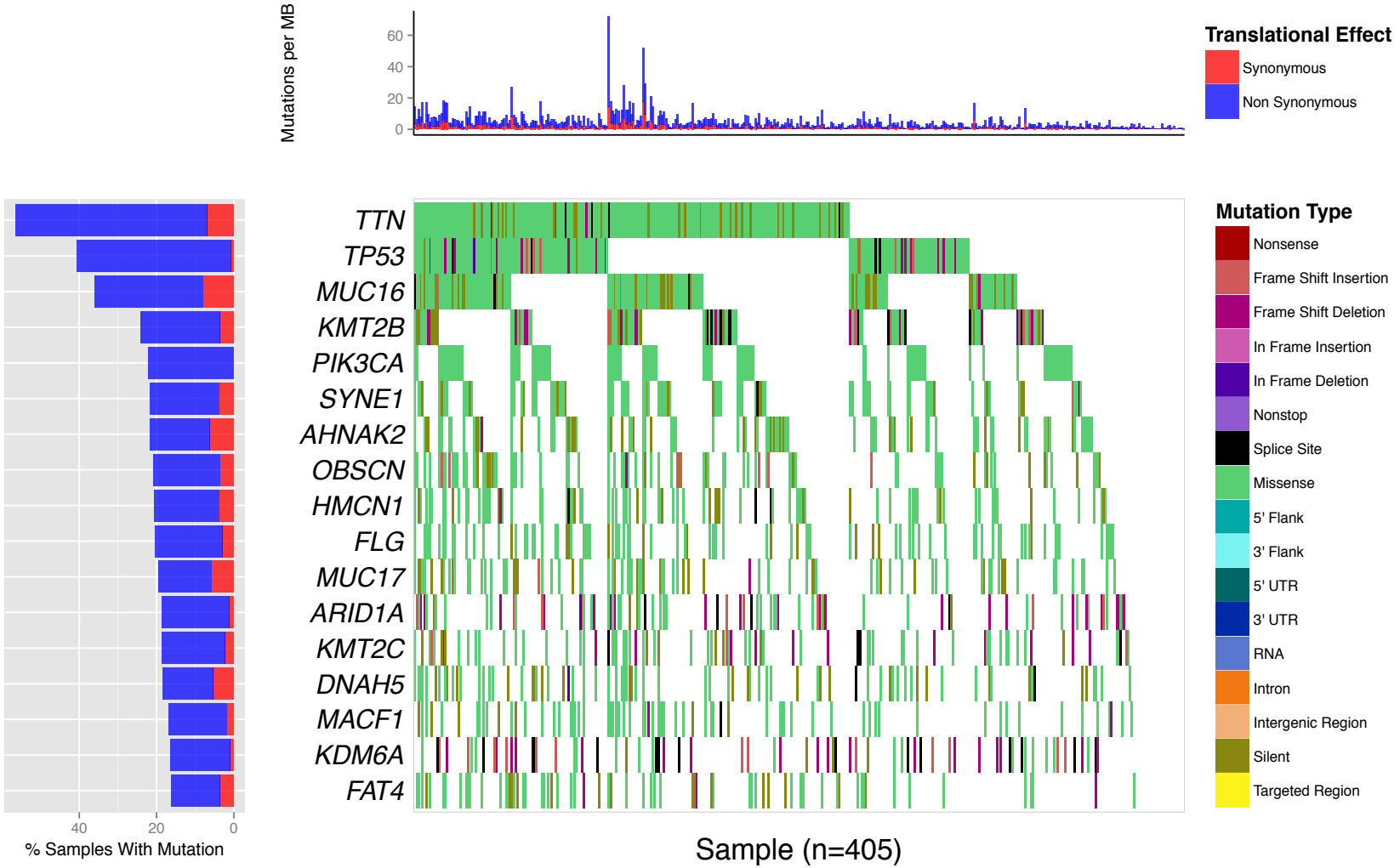


Visualizing copy number variation



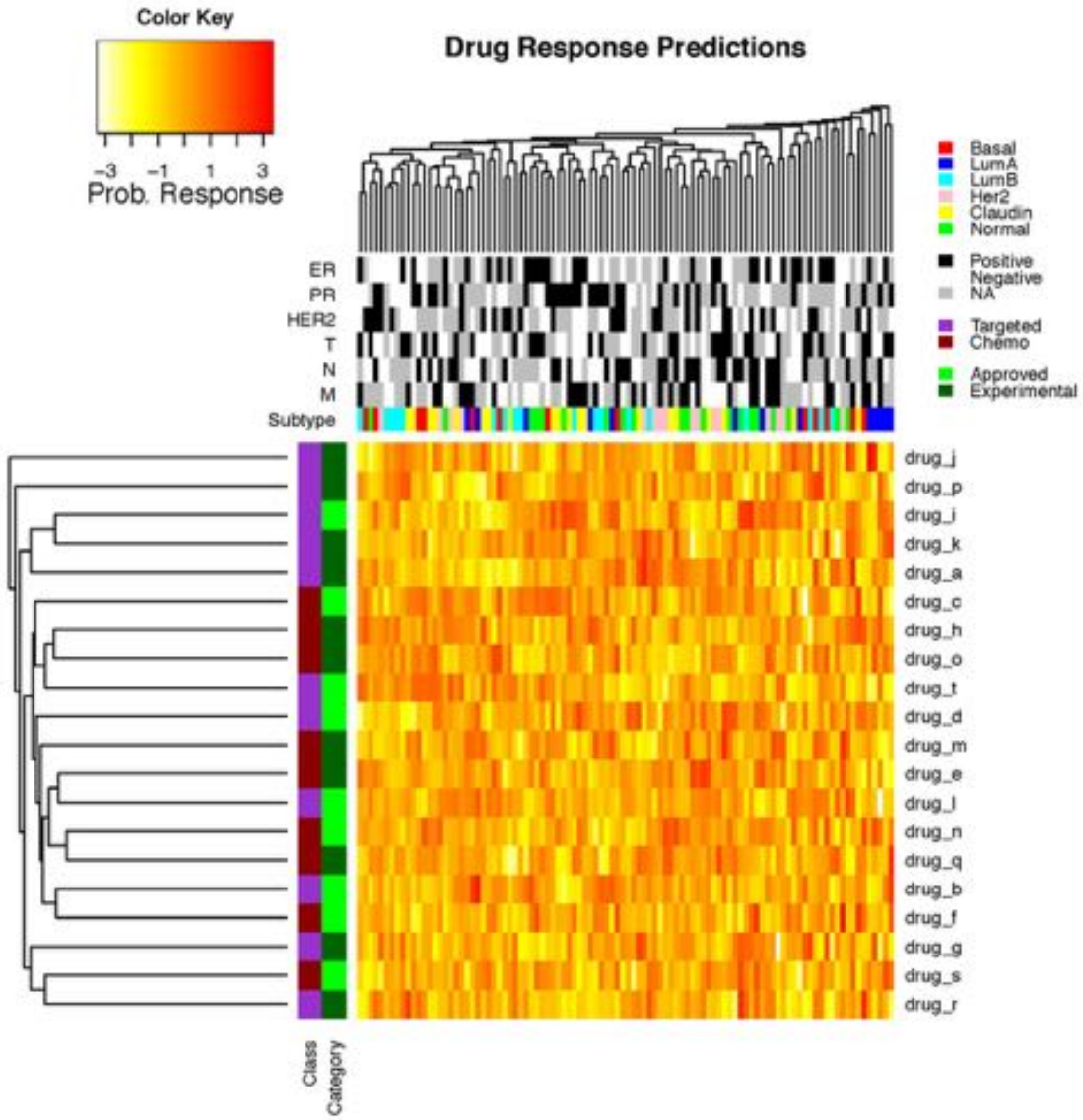
KRAS amplification in a metastatic breast cancer

A ‘waterfall’ plot is one way to visualize the pattern of variant recurrence in a cohort of samples

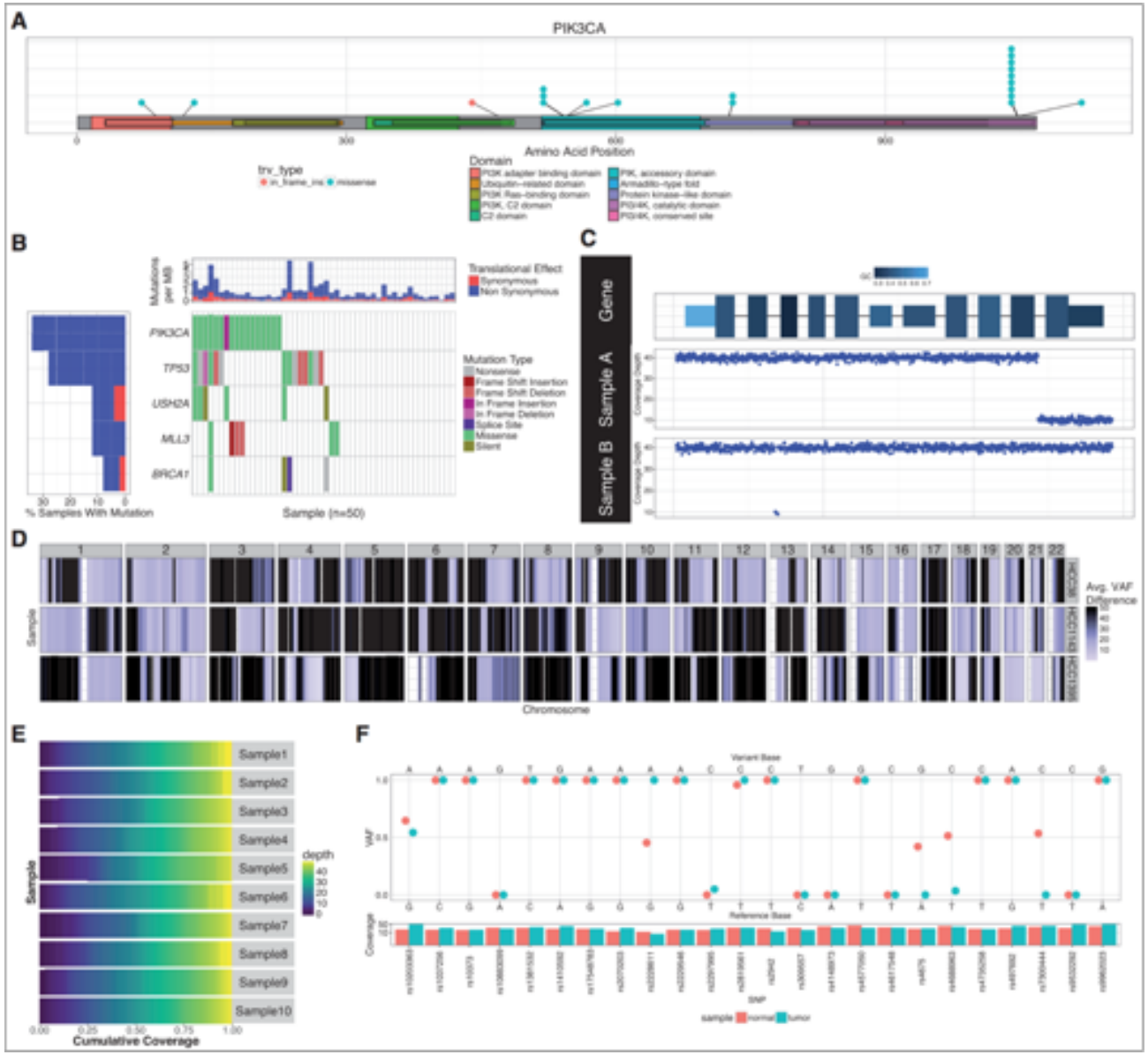


https://github.com/griffithlab/GenVisR

Heatmaps are a common way to simultaneously visualize multiple features of a dataset

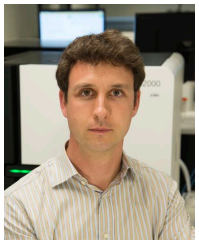


GenVisR was created to help others make common genomic visualizations



Acknowledgements: Griffith Lab group members

Malachi Griffith



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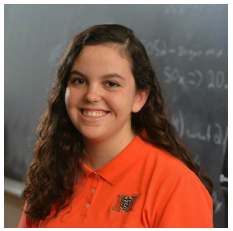

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Regtools/pVA C-seq

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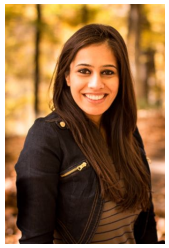
Lymphoma

Felicia Gomez

pVAC-seq



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CIViC/Lymphom a

Kilanin Krysiak

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Lynzey Kujan

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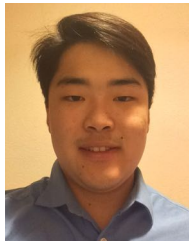

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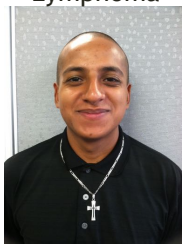

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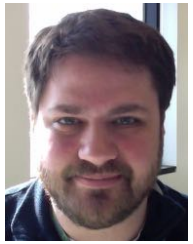
CIViC/Komen

Nick Spies

HCC/SCLC

Lee Trani

DGIdb
SCLC



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Introduction to GenViz course site

www.genviz.org