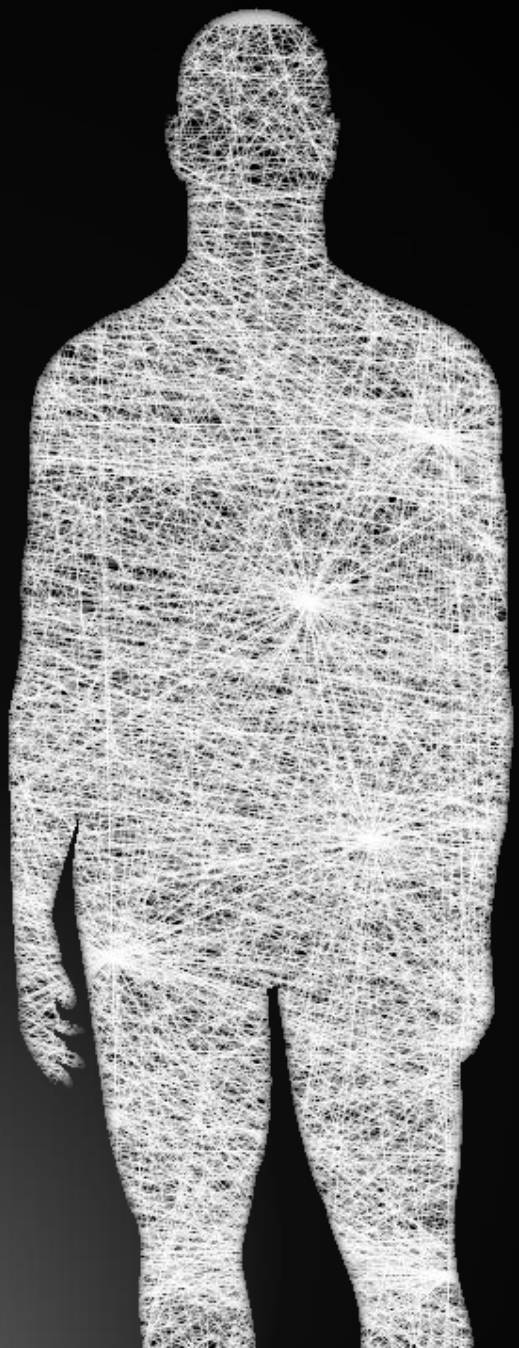
Case Study: Next-Generation Sequencing Implementation for Precision Oncology Testing



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Portland, OR USA

Learning Objectives

- Describe the decision making process in deciding whether to implement next-generation sequencing in a clinical pathology lab setting
- Identify the variety of testing strategies and chemistries available
- Review example case studies where NGS is uniquely suited to provide novel clinical insights

Background about our lab

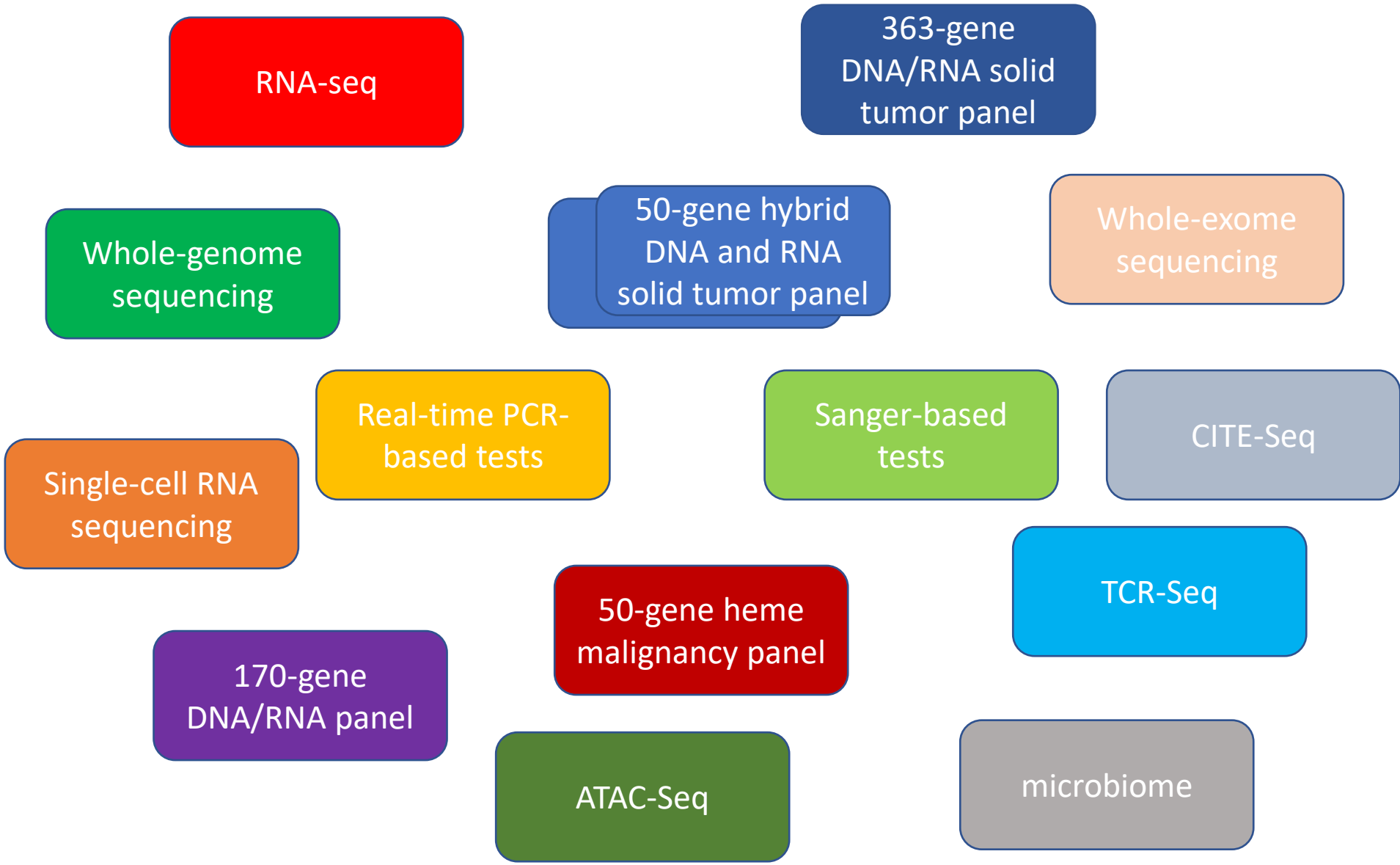
- Serves a community hospital system (now 50+ hospitals)
- Also serving a cancer research institute (Earle A Chiles Research Institute)

Background

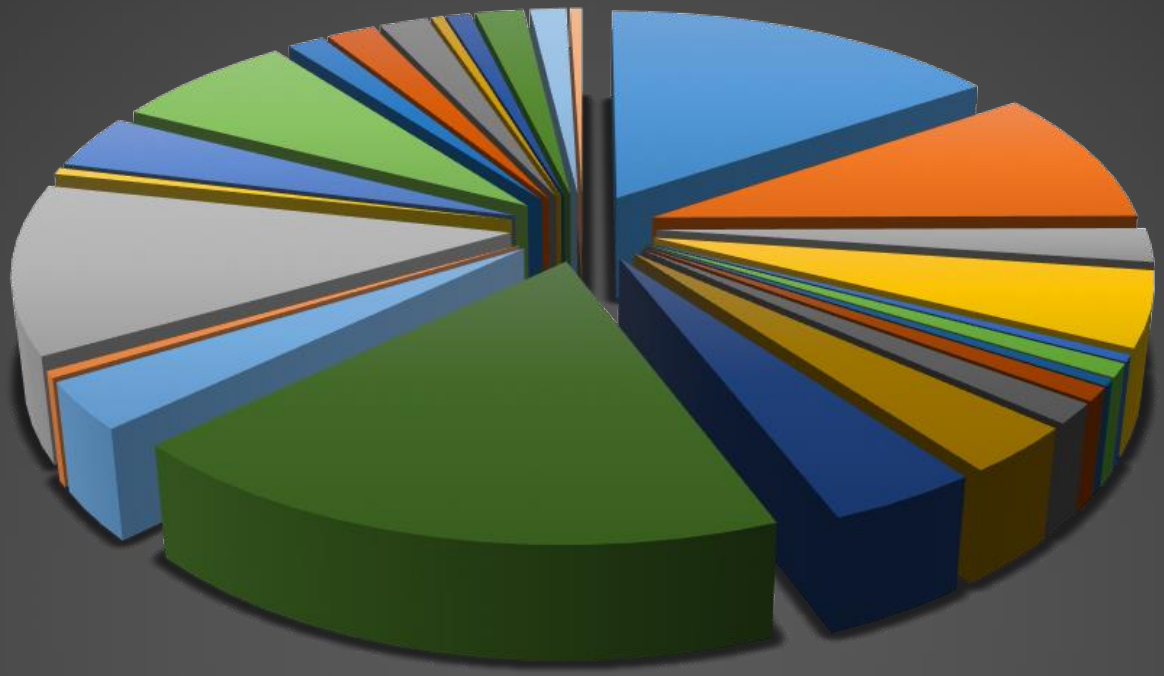
Our molecular genomics laboratory:

- **Began NGS testing in 2015**
- **Housed within the larger clinical testing laboratory**
- **Affiliated with our Pathology Department**

Lab services have evolved over time



Distribution of tumors tested: 363-gene panel



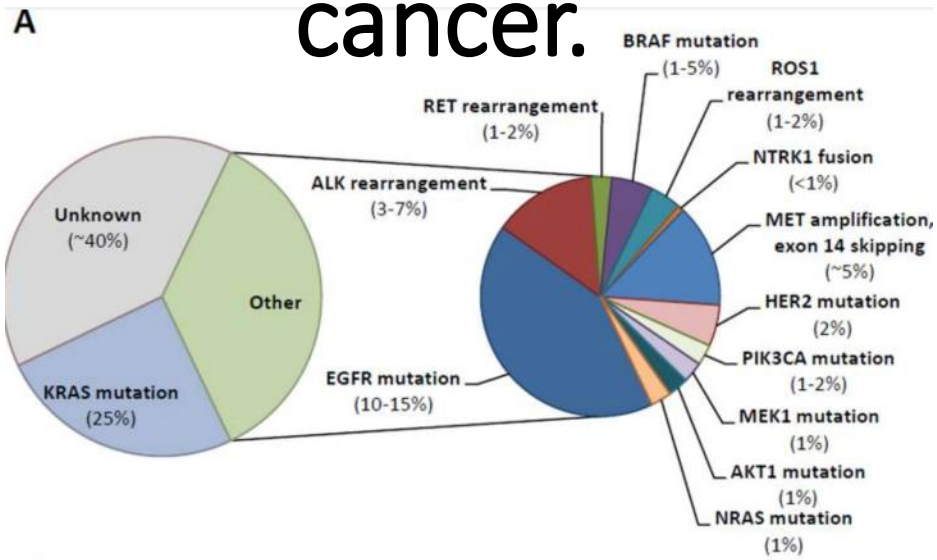
Breast carcinoma
Cholangiocarcinoma
Duodenal adenocarcinoma
Ependymoma
Gastric carcinoma
Glioma
Lung adenocarcinoma
Melanoma
Ovarian serous carcinoma
Prostate carcinoma
Salivary gland carcinoma
Thymoma
Urothelial carcinoma

Cancer, other
Colorectal carcinoma
Endometrial carcinoma
Esophageal carcinoma
Glioblastoma
Head and neck squamous cell carcinoma (HNSCC)
Lung squamous cell carcinoma
Meningioma
Pancreatic carcinoma
Renal cell carcinoma
Testicular cancer
Thyroid carcinoma
Uterine carcinoma

Why NGS testing for somatic cancer?

Top therapeutic mutation targets in lung cancer.

A



B

INHIBITORS			
EGFR	**ALK**	**ROS**	**RET**
Erlotinib*	Crizotinib*	Crizotinib*	Alectinib
Gefitinib*	Ceritinib*	Ceritinib	Cabozantinib
Afatinib*	Alectinib*	Lorlatinib	Vandetanib
Osimertinib*	Lorlatinib	Cabozantinib	Lenvatinib
Rociletinib	Brigatinib	Foretinib	Apatinib
EGF816	X-396	Entrectinib	Ponatinib
ASP8273	Entrectinib	DS-6051b	Sunitinib
HM61713			Dovitinib
MET	**TRK1**	**HER2**	**BRAF/MEK**
Crizotinib	Entrectinib	Afatinib	Vemurafenib
Tivantinib	LOXO-101	Dacomitinib	Dabrafenib
Cabozantinib	DS-6051b	Neratinib	Trametinib
Foretinib		Lapatinib	Selumetinib
Volitinib		Pyrotinib	

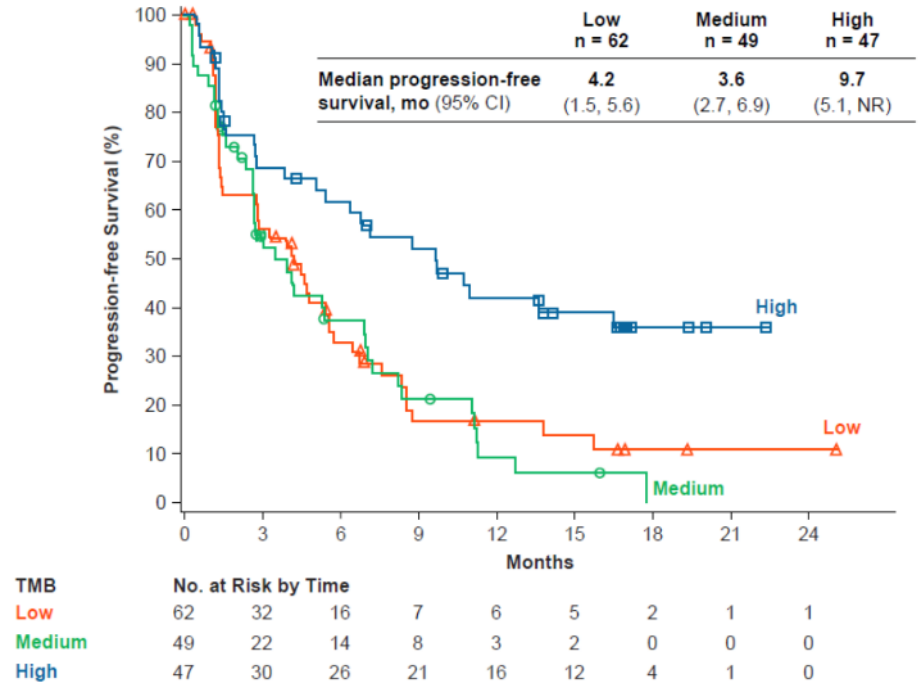
Lin and Shaw, Trends Cancer 2016

The growing field of immuno-oncology is intrinsically linked to genomics

- The success of Tumor Mutational Burden (TMB; # of mutations per megabase) as a biomarker for I-O therapy.

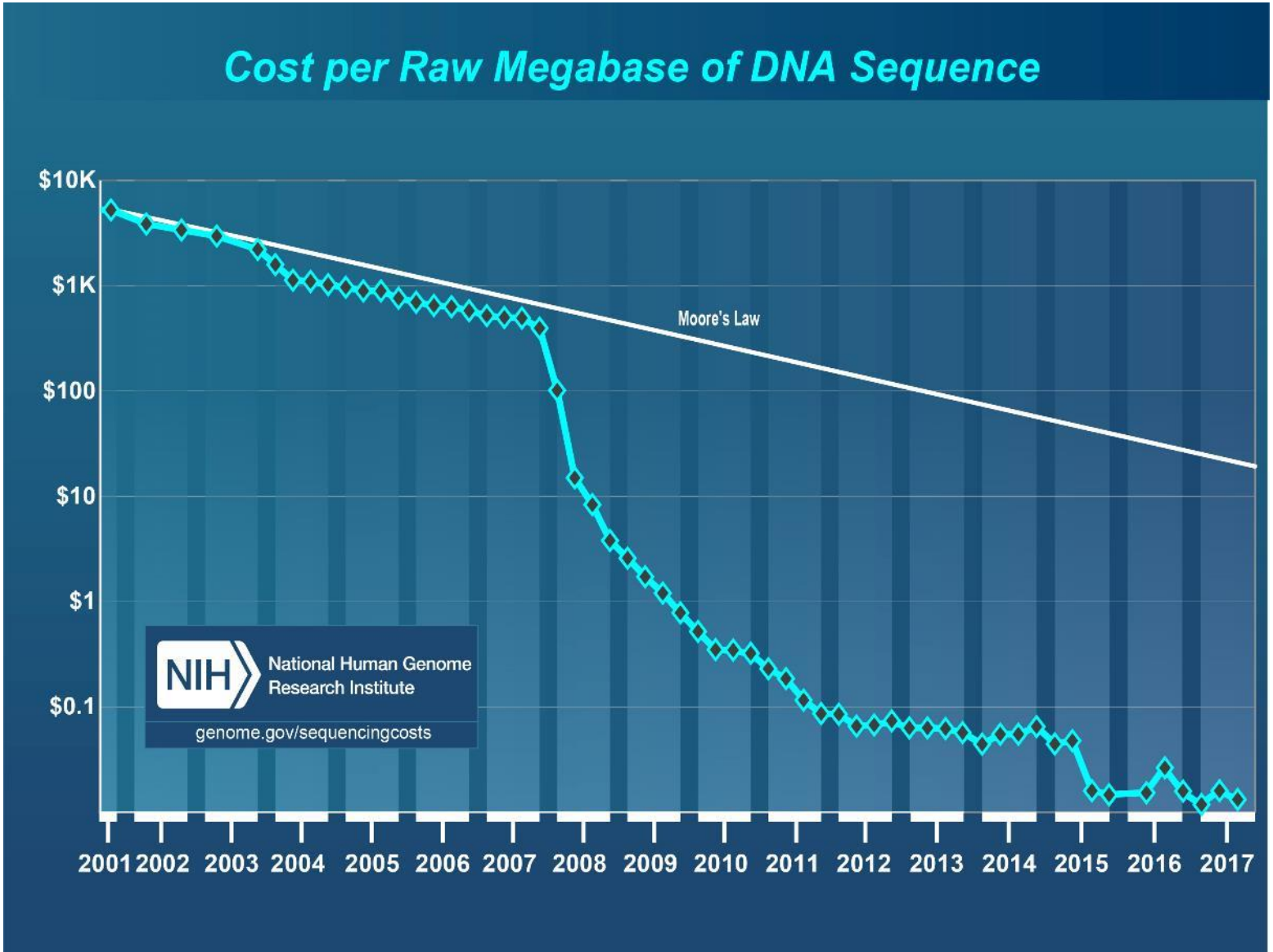
- These successes have also required an expansion in the percent of the genome we test.

Response to anti-PD1 in Lung Cancer for TMB High, Medium and Low cases



Carbone *et al* NEJM 2017

The good news: sequencing has never been more affordable



Why bring NGS in-house versus external testing providers

- Complete flexibility over the content (gene list, chemistry, methodology, reporting).
- Access to complete datasets for research and reanalysis (fastqs, bams etc.).
- NGS is an integral part of research biomedicine.

Which sequencing platform should I choose?

?

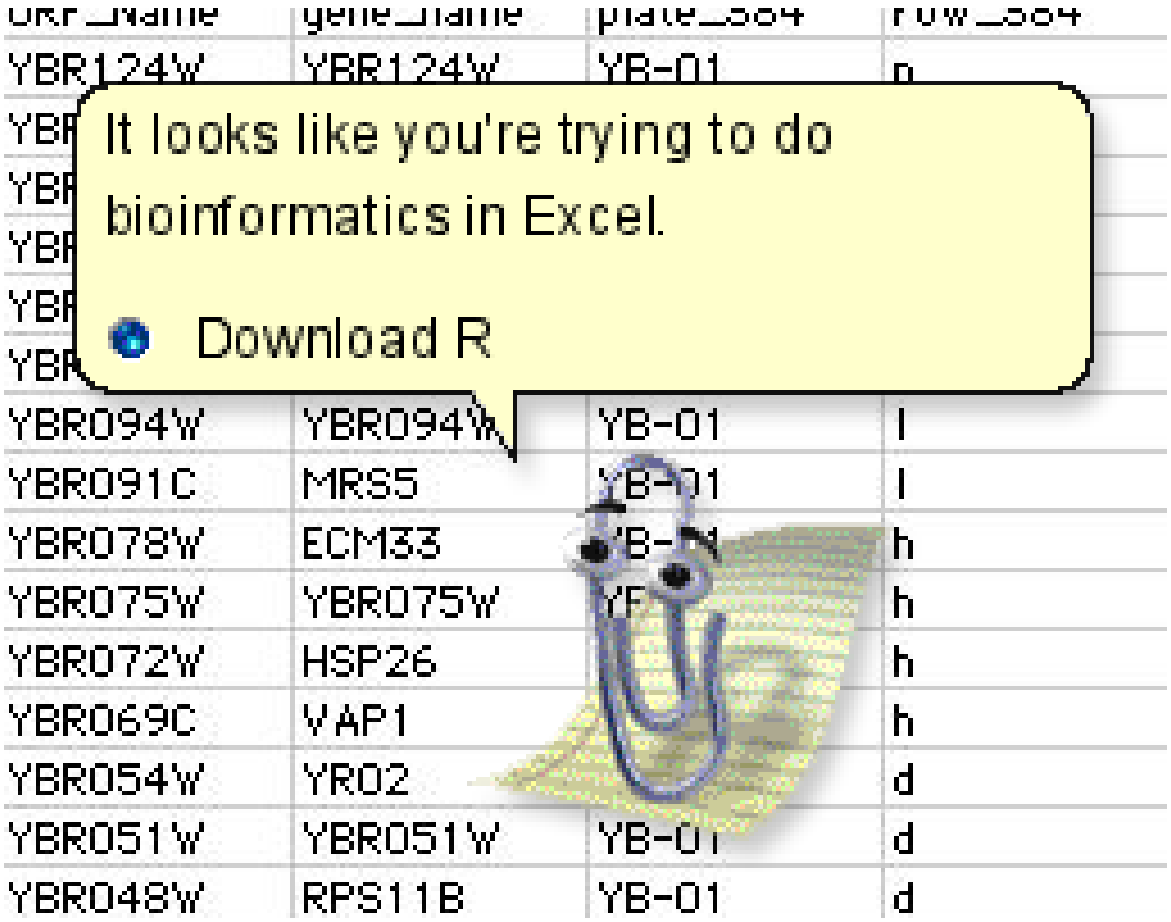
Considerations when choosing NGS platform(s)

1. What is your expected patient test volume?
2. Percentage of the genome that your test(s) will interrogate (e.g. number of Mb per sample)?
3. How fast can you deliver results?

Solid answers to these questions can help to narrow down the platform of choice.

Ok, you've generated data. So now what?

Bioinformatics!



Lots of options here as well

- End-to-end vendor pipelines
- Build your own pipelines
- Local storage and compute vs cloud

The final piece: Interpretation and Reporting

Providence St. Joseph Health

PATIENT INFORMATION

Patient Name: Example Patient Two
Date of Birth: 01/01/1901
Gender: M
MRN: 200000000
Referring Physician: Dr. Prov Portland

Referring Pathologist: Dr. Prov Portland
Accession Number: 17-000-00000
Specimen Type: FFPE
Tissue Type: Omentum
Surgical Path. Case Number: PS-17-000000, A1

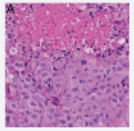
Indication: Melanoma metastatic
Date Collected: 01/01/2018
Date Ordered: 01/01/2018

TRISEQ CLINICAL SEQUENCING PANEL - FINAL REPORT

Report Summary

1 Genomic Findings | 4 FDA approved in patient's tumor type | 0 FDA approved in other tumor types | 5 Clinical Trials

Tissue Pathology



REPORT DETAILS

Genomic Variant	Interpretation Summary	FDA Approved therapies in patients tumor type	FDA Approved therapies in other tumor types	Clinical Trials
BRAF c.1799T>A: p.V600E	**BRAF-V600E is an activating mutation.** BRAF encodes the signaling protein Braf, which is downstream of Ras and activates the MAPK pathway (21447722). Activating mutations in BRAF may predict sensitivity to Raf or MEK inhibitors, some of which have been FDA-approved for certain indications (16273091, 20818844, 22723336, 23020132, 22735384, 23435872, 22663011, 27283860).	YES Vemurafenib, Trametinib, Dabrafenib, Cobimetinib	NO	YES See clinical trials sections

Summary:

- It has never been easier to bring NGS testing on-line in your lab/institute.
- New targeted therapy and immune-oncology developments will further increase the value of these results for oncology patients.

Case study: A prototypical NGS application

Case study - 50 y.o. female

- Presented to clinic with a range of symptoms
  - Facial numbness
  - Partial hearing loss
  - Persistent cough
- Brain MRI, chest CT were performed
- **Diagnosis of primary lung cancer with brain metastasis**
- Median survival for this diagnosis historically has been **5-6 months** (Ali *et al.* Curr. Oncol. 2013).

Case study (continued):

- Lung biopsy was performed.
- Tissue preserved in formalin, embedded in paraffin wax (FFPE).
- Sections cut and affixed to microscope slides for review by pathologist.
- Genomic sequencing was ordered.
- DNA and RNA were extracted from tissue sample and sequenced.

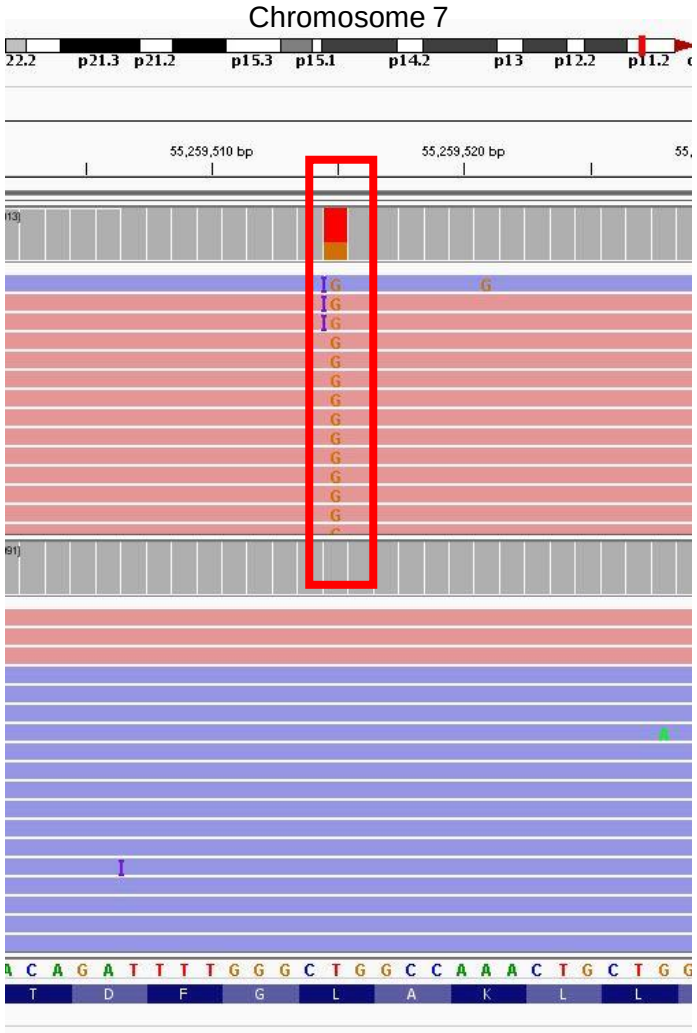
Case study (continued):

Sequencing result:

- *EGFR* c.2573T>G: p.L858R

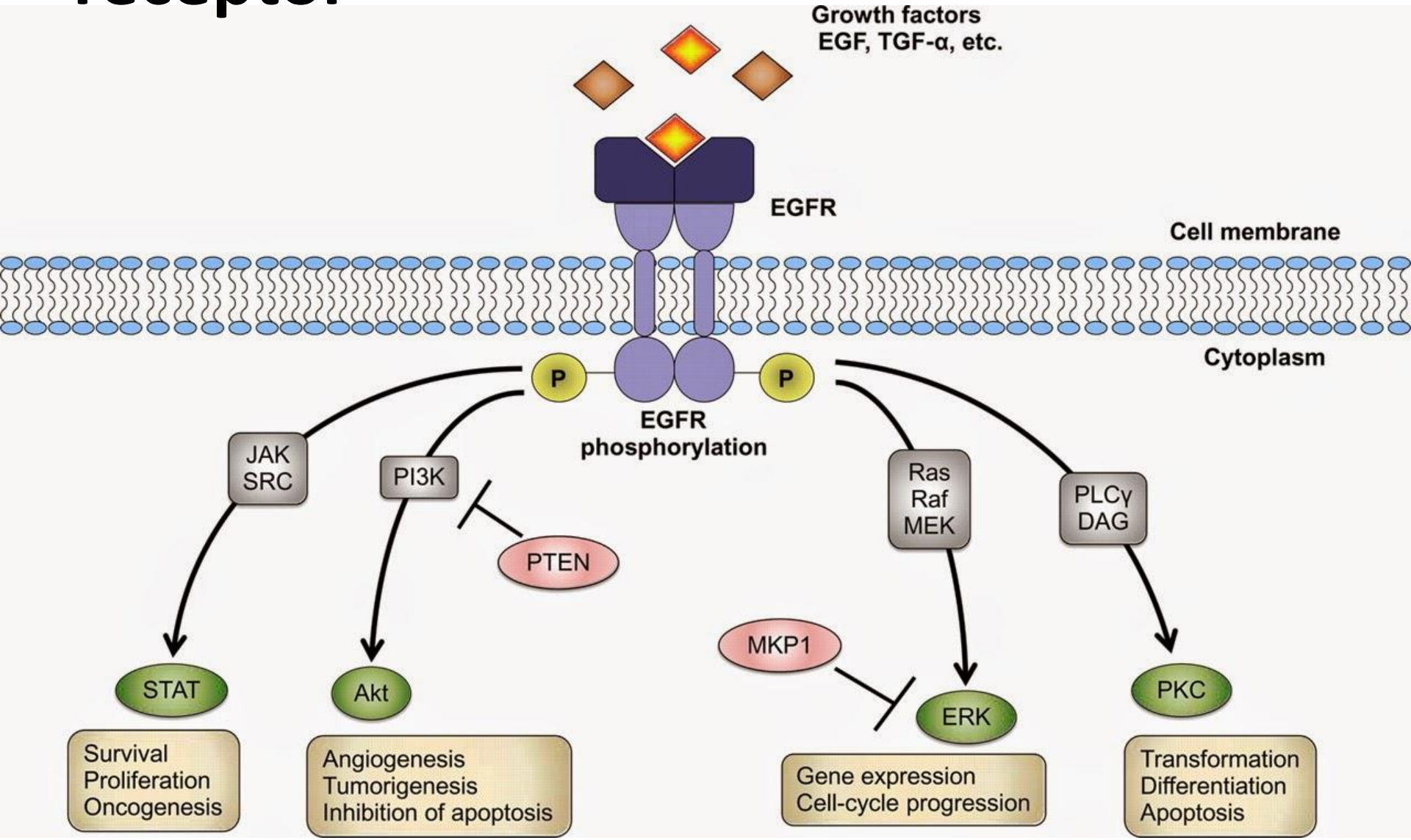
TUMOR SAMPLE

NEGATIVE CONTROL



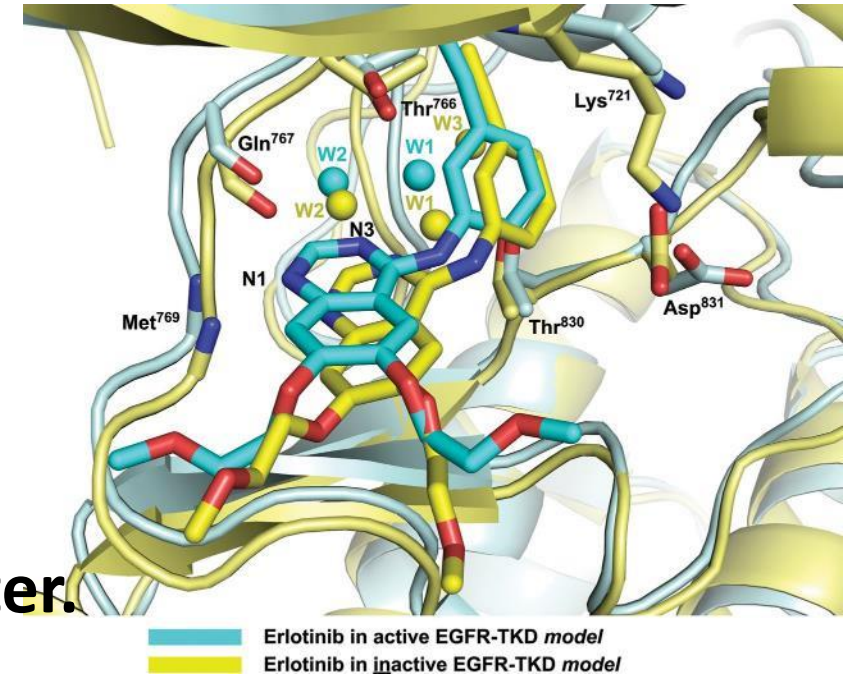
EGFR

EGFR – epidermal growth factor receptor



Case study (continued):

- Patient was put on a therapy targeting EGFR L858R.
- Erlotinib is a tyrosine kinase inhibitor (TKI).
- Tumors exhibited rapid reduction in size.
- **Patient still alive ~2 years later.**



Park *et al.* Biochem. Journal 2012

Survival in patients with EGFR-activating mutations (Phase III Data)

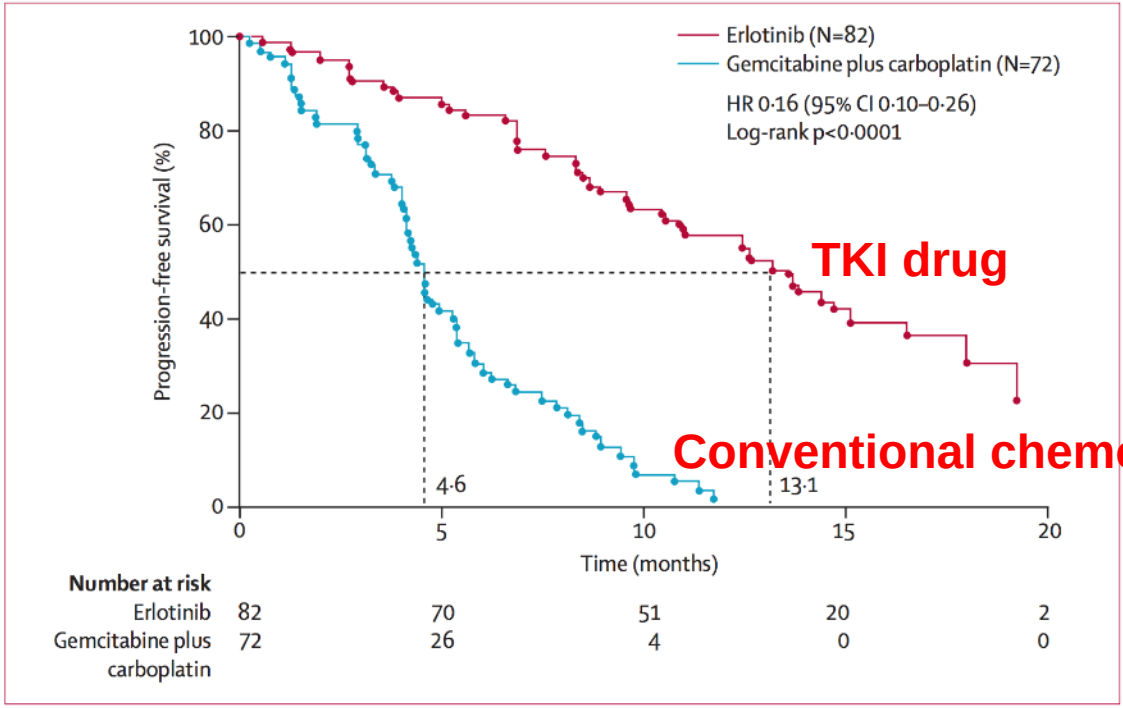


***Figure 2*: Progression-free survival in both treatment groups**
PFS=progression-free survival. HR=hazard ratio.

Zhou *et al.* Lancet Oncol. 2011

Back to our case study:

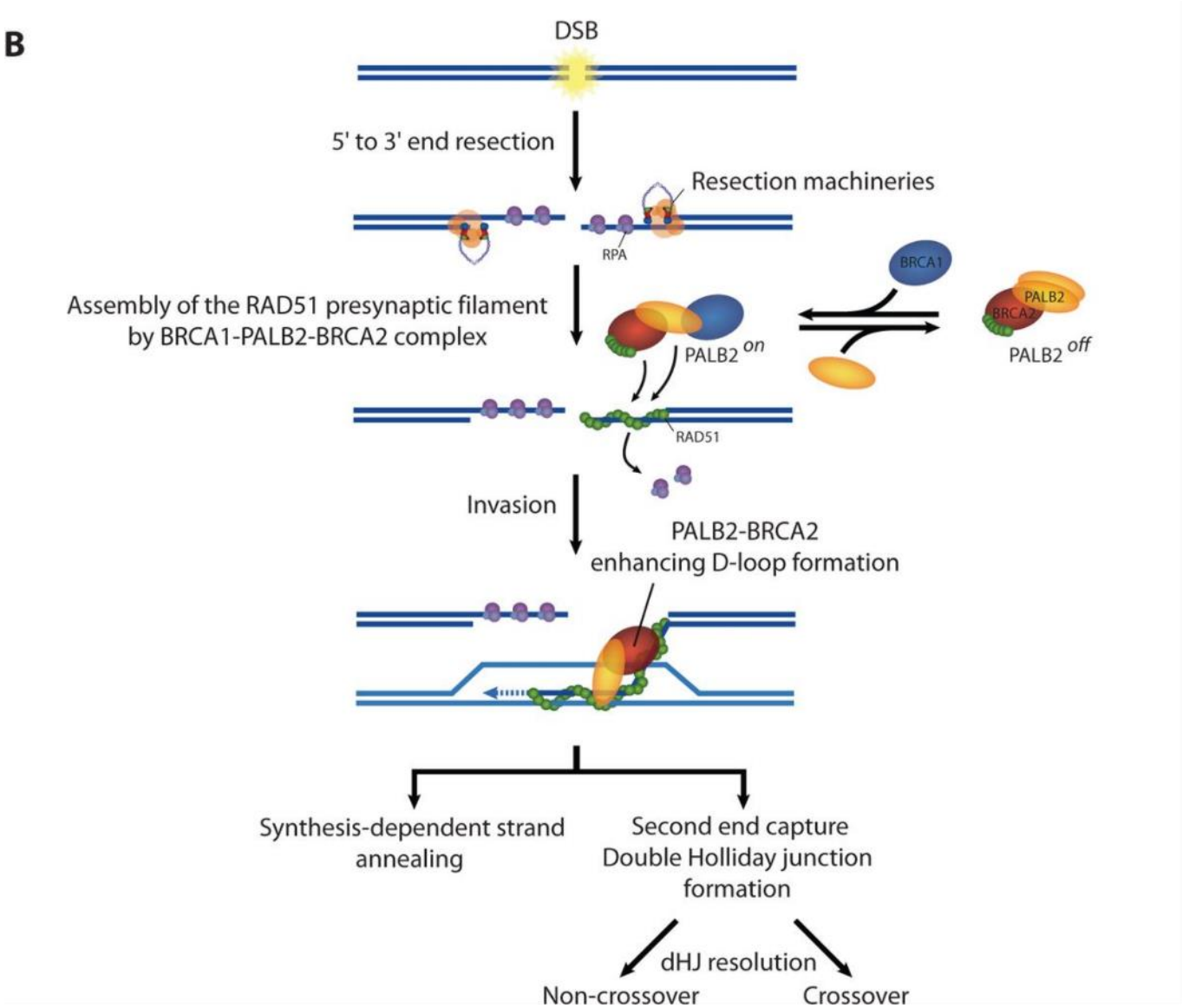
- At ~2 year mark, new scans revealed that patient tumors now progressing again.
- Sequencing of new biopsy sample reveals the presence of EGFR T790M mutation.
- T790M is a common acquired resistance mechanism for TKI therapies.
- What to do now? Immunotherapy?

Case study: The atypical case

Case study 2 – 38 y.o. female

- Stage IIIA triple negative metastatic breast cancer.
- Due to family history and age of diagnosis, patient was referred to genetic counseling.
- Identification of **pathogenic *germline PALB2* 4-bp frameshift deletion**.
- Carboplatin added to treatment plan; tumor exhibited resistance to carbo.
- Tumor and germline whole exome sequencing performed.

PALB2 forms complex with BRCA1/2 in DNA repair.



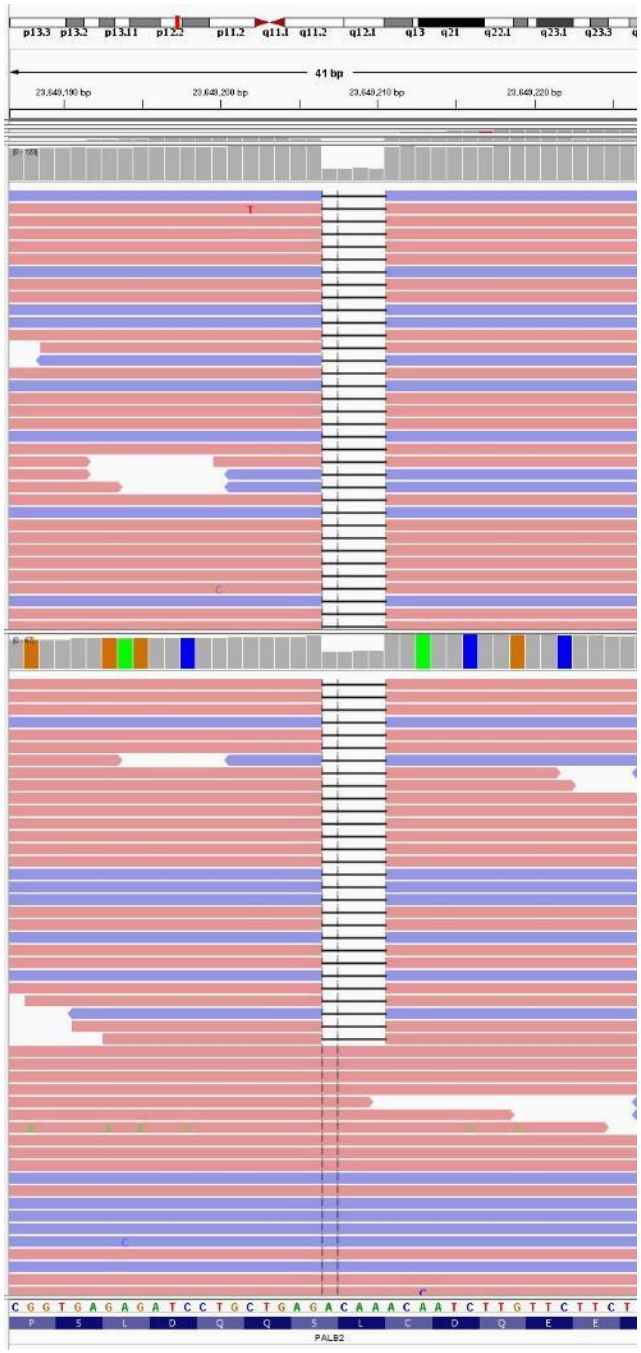
Buisson and Masson, PNAS 2012

Confirmation of 4-bp *PALB2* frameshift deletion in both germline and carbo-resistant tumor.

Tumor

Formal HGVS indication:
PALB2
c.172_175delTTGT:p.Gln60fs

Germline



Identification of novel 5’ 8-bp deletion in tumor only.

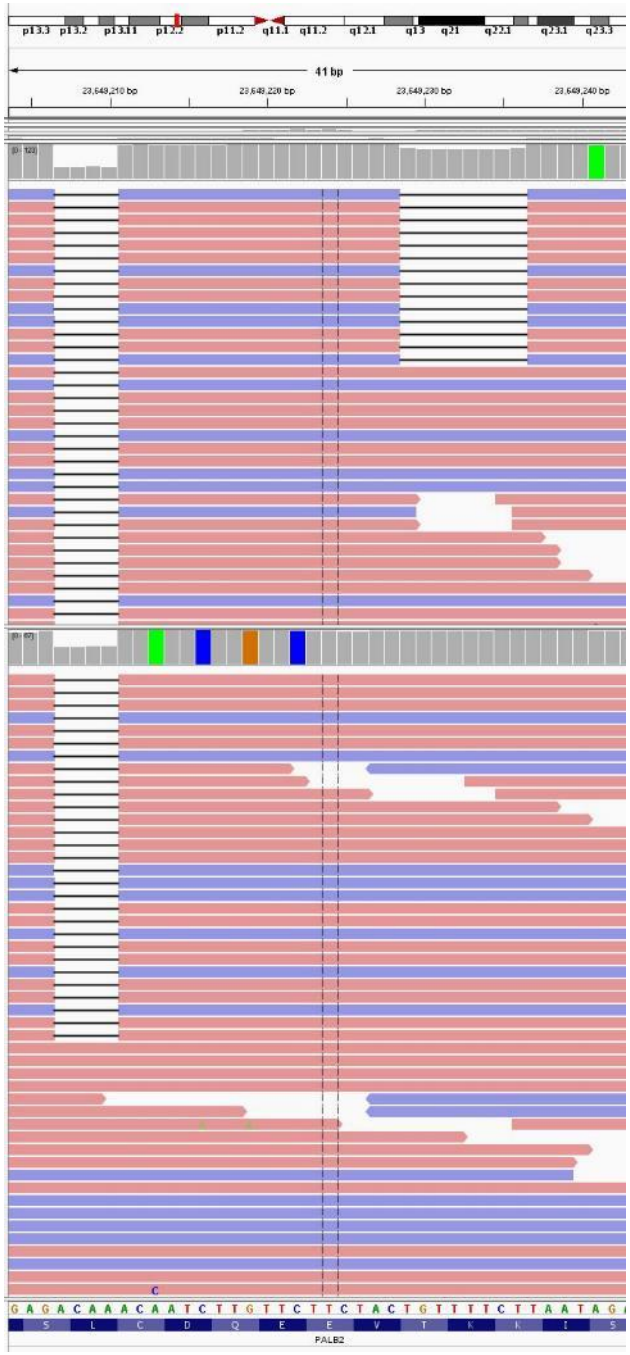
- Deletion restores *PALB2* reading frame in the tumor.

Tumor

Formal HGVS indication:
PALB2 c.172_175delTTGT:p.Gln60fs

PALB2 c.[146_153del; c.172_175del]:
p.Lys49_Cys57delinsSerArgArgThrArg

Germline



Restoring mutations have been identified as mechanism for resistance in other *BRCA* complex genes.

Priority Report

Secondary *BRCA1* Mutations in *BRCA1*-Mutated Ovarian Carcinomas with Platinum Resistance

Elizabeth M. Swisher,[1,2] Wataru Sakai,[3,4] Beth Y. Karlan,[5] Kaitlyn Wurz,[1,2] Nicole Urban,[4] and Toshiyasu Taniguchi[3,4]

Departments of [1]Obstetrics and Gynecology and [2]Medicine, University of Wa
Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington; an
Research Institute, Los Angeles, California

Abstract

**Although ovarian carcinomas with mutated *BRCA1* or *
are sensitive to platinum compounds, such carci
eventually develop platinum resistance. Previously, we s
that acquired resistance to cisplatin in *BRCA2*-m
tumors can be mediated by secondary intragenic mut**

Cancer Res. 2008

nature Vol 451|28 February 2008|doi:10.1038/nature06633

LETTERS

Secondary mutations as a mechanism of cisplatin resistance in *BRCA2*-mutated cancers

Wataru Sakai[1,2], Elizabeth M. Swisher[3,4], Beth Y. Karlan[5], Mukesh K. Agarwal[6], Jake Higgins[4,7], Cynthia Friedman[1], Emily Villegas[1,2], Céline Jacquemont[1,2], Daniel J. Farrugia[6], Fergus J. Couch[6], Nicole Urban[2] & Toshiyasu Taniguchi[1,2]

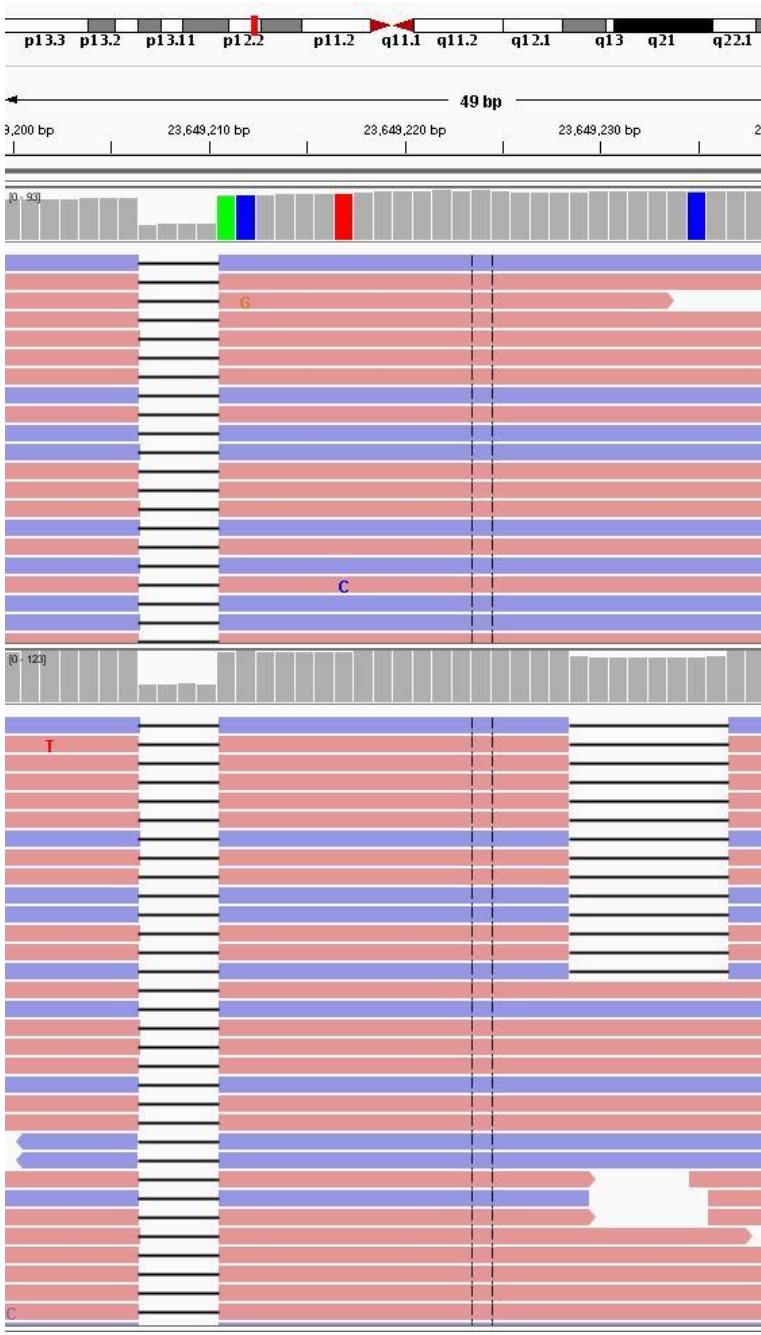
Nature 2008

The original pre-carbo core biopsy was obtained and exome sequencing was performed.

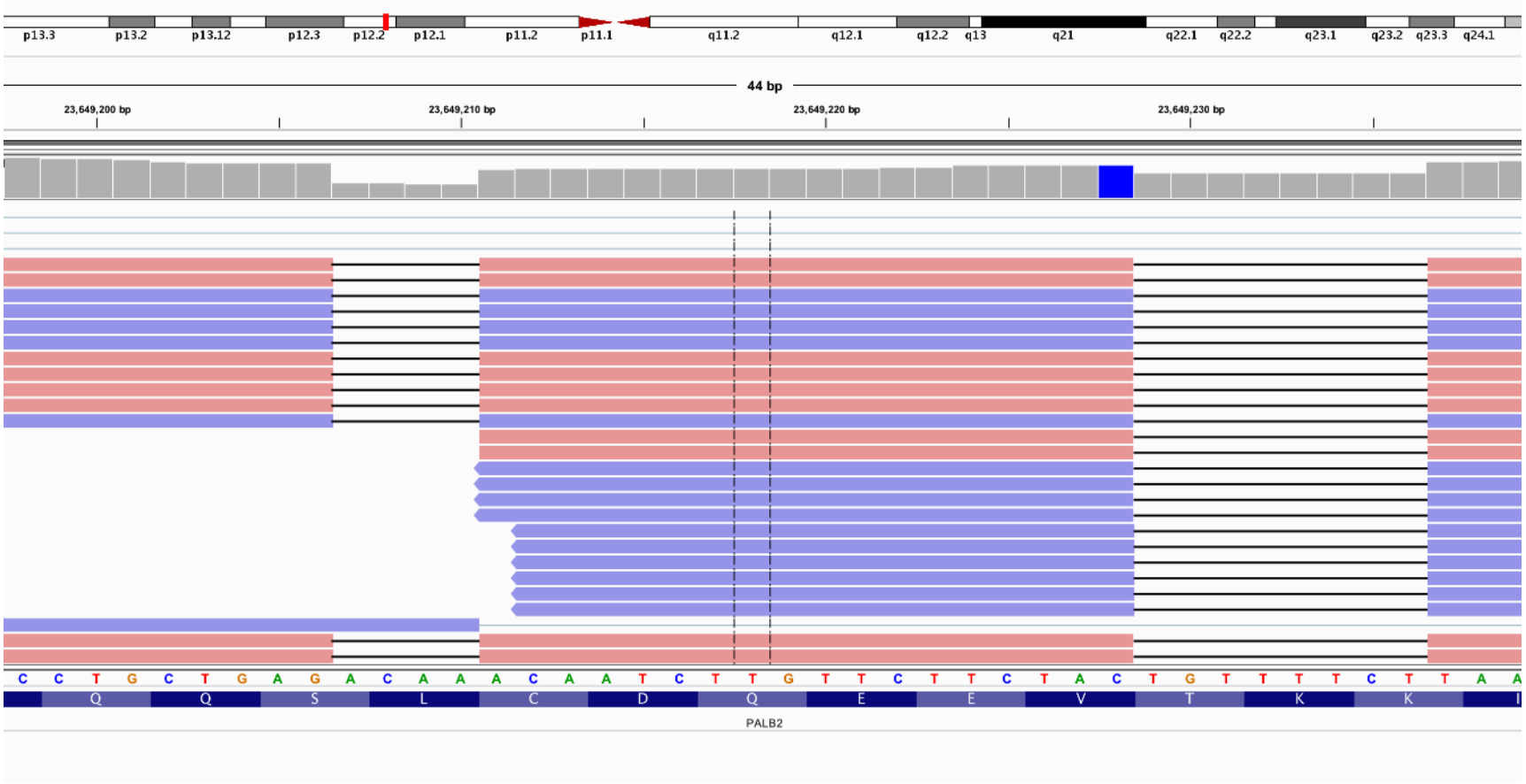
- Secondary *PALB2* reversion mutation is only detected in the post-carboplatin sample.
- *PALB2* frame restoration likely occurred as resistance mechanism to carbo.

Pre-carbo

Post-carbo



The frameshift and reversion are present in the RNA-seq data as well.



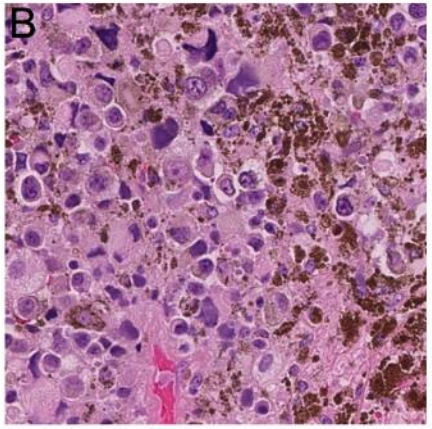
RNA-seq data also confirm LOH in original *PALB2* frameshift.

Follow-up for *PALB2* restoration case:

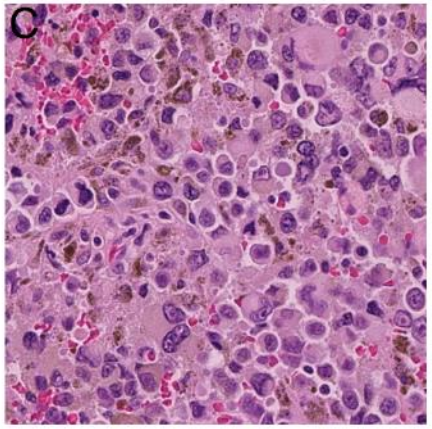
- Patient unlikely to benefit from PARP inhibitor therapy
- Patient considering immunotherapy trials

Case study: Immunotherapy considerations

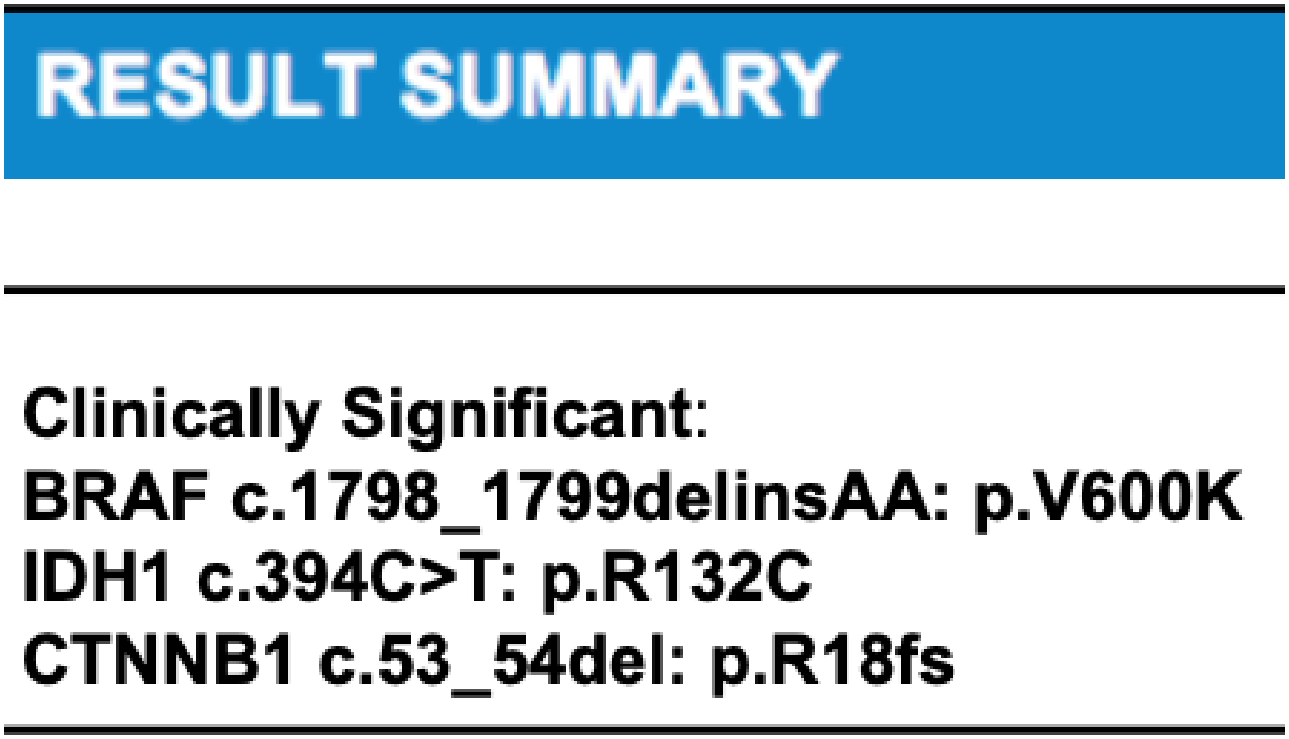
Metastatic melanoma patient – 71 y.o. male



- Patient with history of metastatic melanoma (primary lesion not known).
- Prior lesions:
  - 15 years ago: right upper back lesion
  - 8 years ago: new back lesion distal site
  - Current lesion: adrenal resection



Metastatic melanoma case - initial 50-gene targeted hotspot panel sequencing results



- BRAF inhibitor therapy an option
- Patient also considering immunotherapy trials
- Larger sequencing panel was utilized.

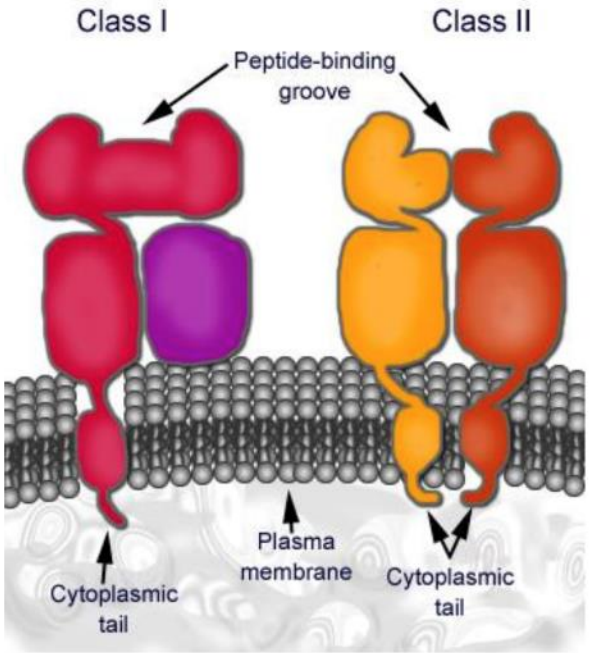
Sequencing with 363-gene panel and whole exome

- 43 mutations found in the NGS panel, 8 of known clinical significance.
- **Tumor mutational burden analysis on exome is clear TMB-high (>30 mut/Mb).**

RESULT SUMMARY
Genomic Alterations Detected
Clinically Significant: B2M c.275_276delCC:p.Pro92fs, BCOR c.4038_4039delAG:p.Glu1348fs, BRAF c.1798_1799delGTinsAA:p.Val600Lys, DNMT3A c.2207G>A:p.Arg736His, IDH1 c.394C>T:p.Arg132Cys, KMT2B c.7852G>T:p.Glu2618*, SDHA c.712_713delTG:p.Cys238fs, TET2 c.C3820T:p.Gln1274*
Unknown Clinical Significance: ARID5B c.610G>A:p.Asp204Asn, BRCA1 c.2812C>T:p.Pro938Ser, CBLC c.484G>A:p.Glu162Lys, CCND1 c.505C>A:p.Pro169Thr, CTNNB1 c.53_54delGA:p.Arg18fs, DPYD c.1730C>T:p.Ser577Phe, EPHA3 c.709G>A:p.Glu237Lys, EZH2 c.694C>T:p.Pro232Ser, FANCA c.44C>T:p.Pro15Leu, FLT1 c.1123C>T:p.Pro375Ser, GLI3 c.3113C>T:p.Ser1038Phe, IGF1R c.2006C>A:p.Pro669His, IRS2 c.2681C>T:p.Ser894Phe, KMT2A c.7851_7852delCCinsTT:p.ProArg2617ProCys, KMT2B c.5201C>T:p.Ser1734Phe, KMT2B c.7819A>G:p.Ile2607Val, KMT2C c.9605T>G:p.Ile3202Ser, LRP1B c.9458C>T:p.Pro3153Leu, LRP1B c.7658G>A:p.Arg2553Gln, MAP3K9 c.677G>A:p.Gly226Glu, MSH6 c.1006A>G:p.Thr336Ala, MTOR c.3101_3102delTA:p.Ile1034fs, NCOR1 c.775C>G:p.Pro259Ala, NCOR1 c.490C>T:p.Pro164Ser, NTRK2 c.1356T>A:p.Phe452Leu, NTRK3 c.2317C>T:p.Arg773Cys, PRSS1 c.740G>A:p.Ser247Asn, PTEN c.1069C>T:p.Pro357Ser, ROS1 c.3667G>A:p.Val1223Ile, SF3B1 c.1106C>T:p.Thr369Ile, SIN3A c.1048A>T:p.Thr350Ser, SMO c.74A>G:p.Asp25Gly, TAL1 c.991C>T:p.Arg331Trp, TRAF2 c.1497_1498delAG:p.Gly500fs, TSC2 c.3476G>T:p.Arg1159Leu

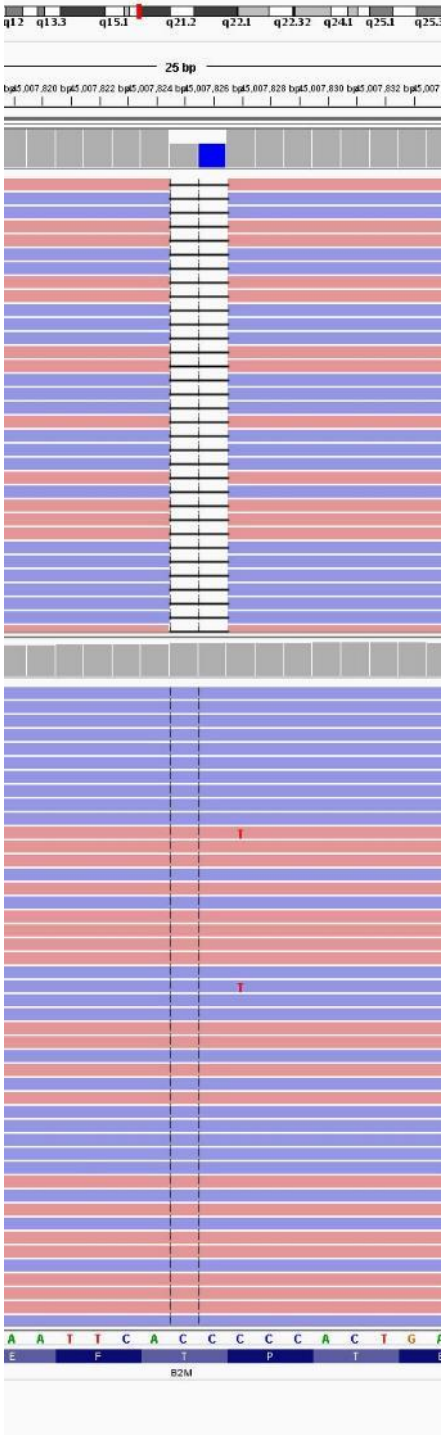
Extensive sequencing panel revealed frameshift mutation in the *B2M* gene (Beta-2-Microglobulin)

- B2M a requirement for MHC class I antigen presentation



2017 sample

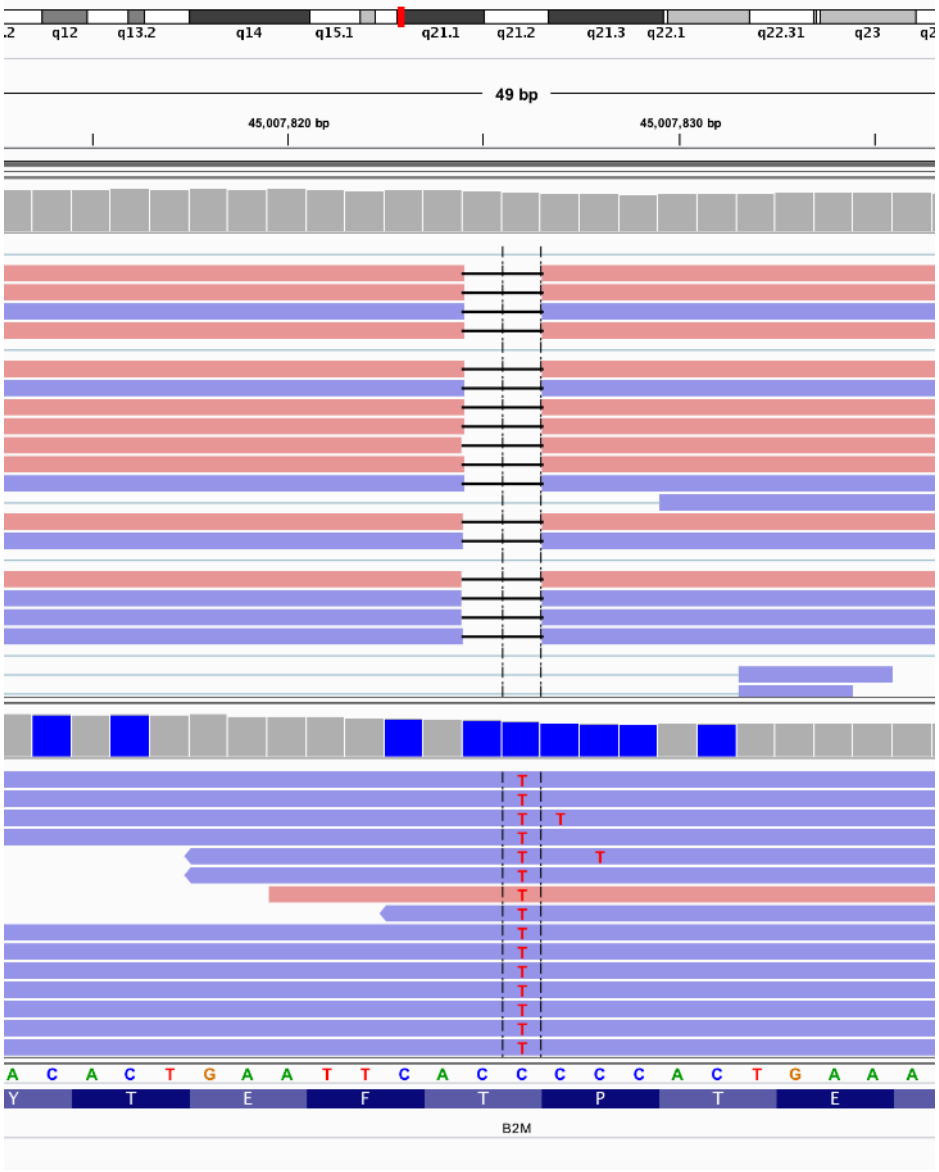
2004 sample



B2M frameshift also detected in RNA

2017 sample

2004 sample



Loss of B2M a recently discovered immunotherapy evasion mechanism in melanoma.

Loss of Functional Beta$_2$-Microglobulin in Metastatic Melanomas From Five Patients Receiving Immunotherapy

Nicholas P. Restifo, Francesco M. Marincola, Yutaka Kawakami, Jeff Taubenberger, John R. Yannelli, and Steven A. Rosenberg

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ORIGINAL ARTICLE

Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma

Jesse M. Zaretsky, B.S., Angel Garcia-Diaz, Ph.D., Daniel S. Shin, M.D., Helena Escuin-Ordinas, Ph.D., Willy Hugo, Ph.D., Siwen Hu-Lieskovan, M.D., Ph.D., Davis Y. Torrejon, M.D., Gabriel Abril-Rodriguez, M.Sc., Salemiz Sandoval, Ph.D., Lucas Barthly, M.Sc., Justin Saco, B.S., Blanca Homet Moreno, M.D., Riccardo Mezzadra, M.Sc., Bartosz Chmielowski, M.D., Ph.D., Kathleen Ruchalski, M.D., I. Peter Shintaku, Ph.D., Phillip J. Sanchez, Ph.D., Cristina Puig-Saus, Ph.D., Grace Cherry, R.N., N.P., Elizabeth Seja, B.A., Xiangju Kong, M.Sc., Jia Pang, B.S., Beata Berent-Maoz, Ph.D., Begoña Comin-Anduix, Ph.D., Thomas G. Graeber, Ph.D., Paul C. Tumeh, M.D., Ton N.M. Schumacher, Ph.D., Roger S. Lo, M.D., Ph.D., and Antoni Ribas, M.D., Ph.D.

N Engl J Med 2016; 375:819-829 | September 1, 2016 | DOI: 10.1056/NEJMoa1604958

Patient unlikely to benefit from immunotherapy

Summary:

- Extended sequencing panels can have a significant impact on treatment decisions
- Routine WES, WGS, RNA-seq likely not far off in clinical practice

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