Misadventures of genomic technology in gynecologic oncology

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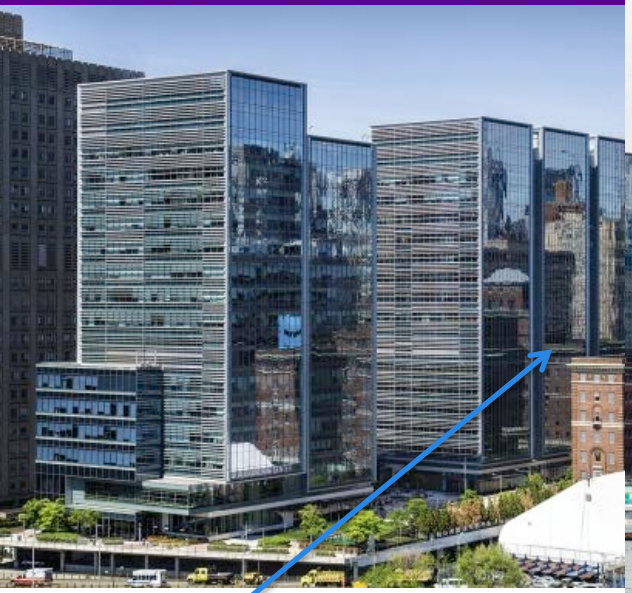
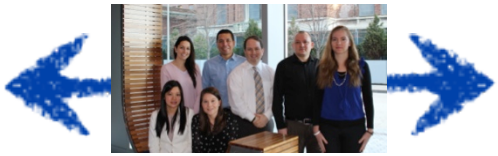
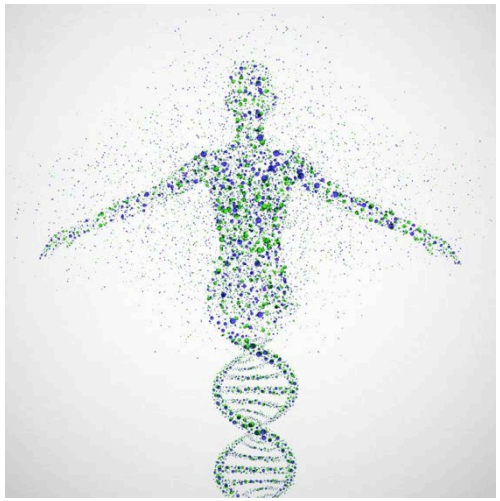


Gyn Res Lab

The Gynecology Research Laboratory studies ovarian and endometrial cancers.



Novel biomarkers



Precision medicine

Rare tumor research

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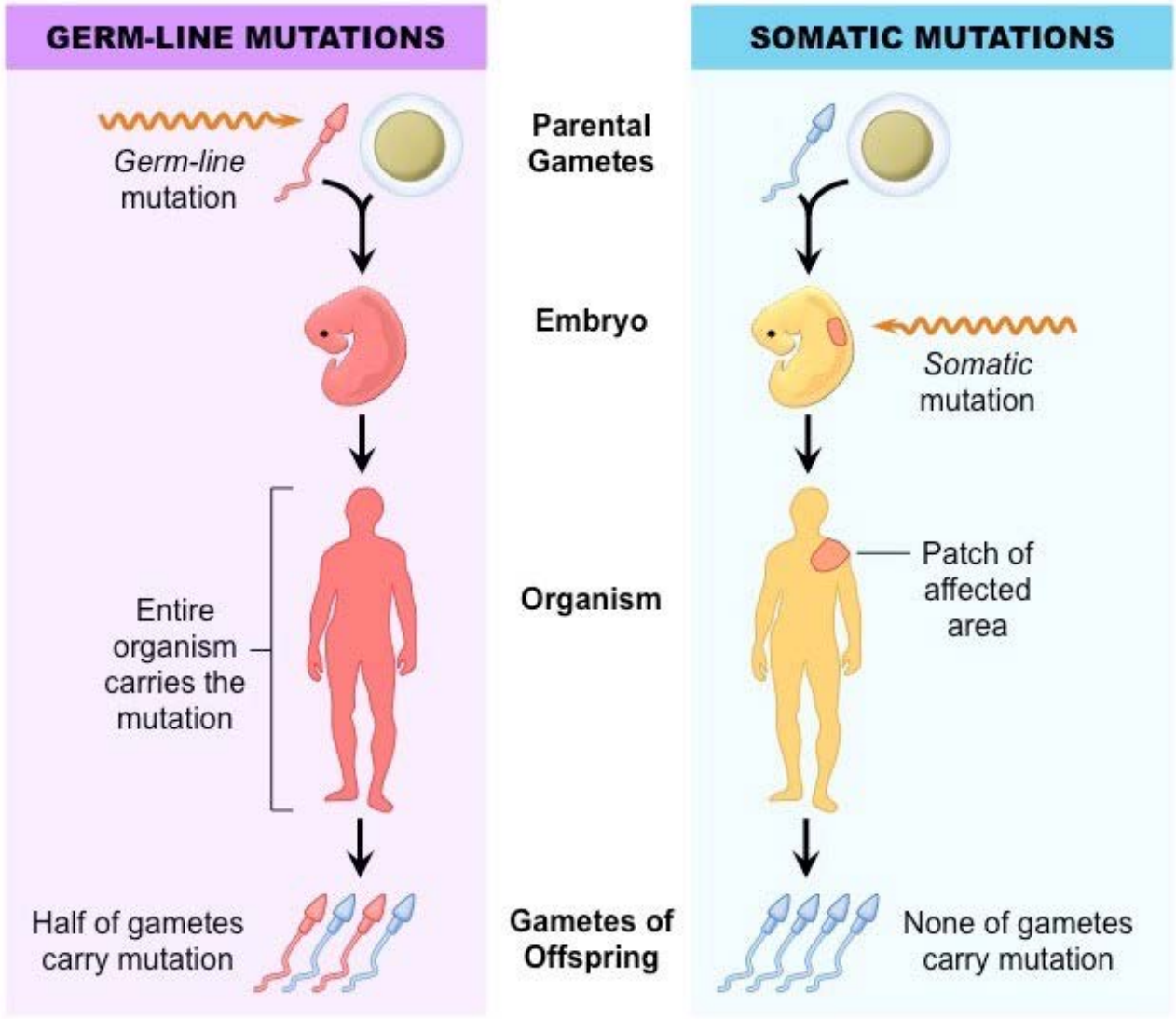
Outline

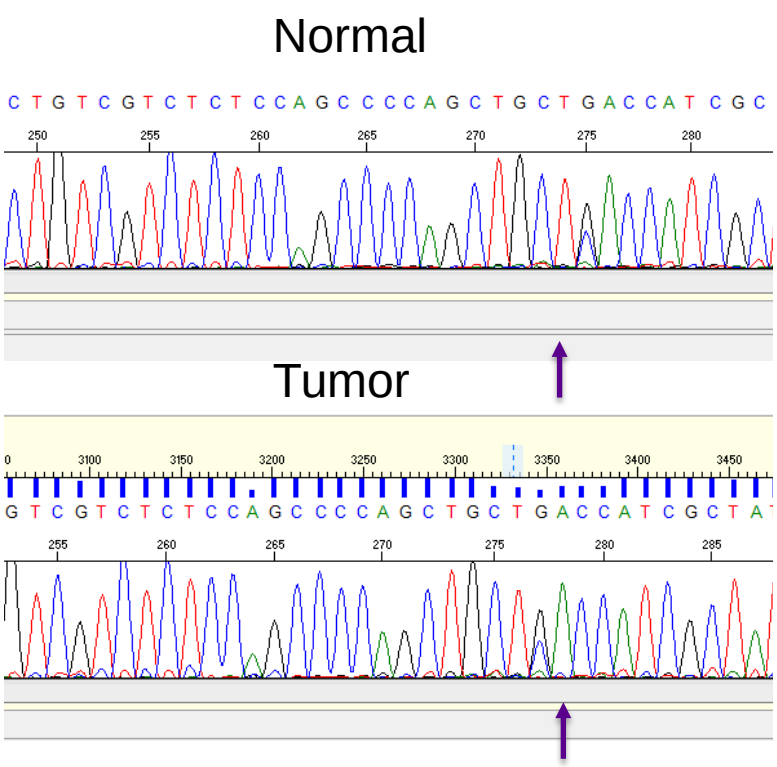
- Genetic 101
- What has genomics done for us to date?
- Available technology
- Unmet needs

Just to be clear….

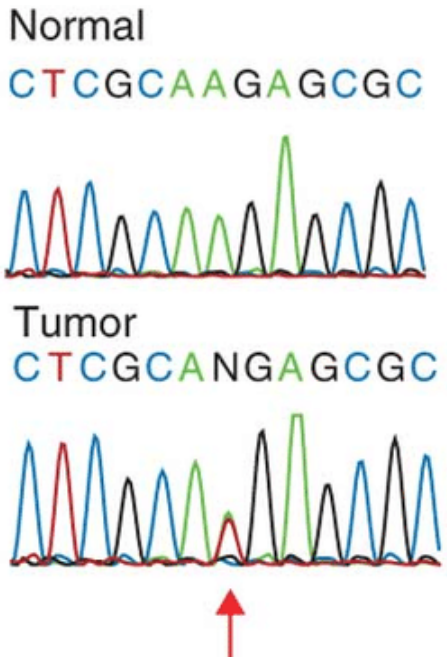


Sanger sequencing – 2X coverage

Germline variant found
in normal and tumor

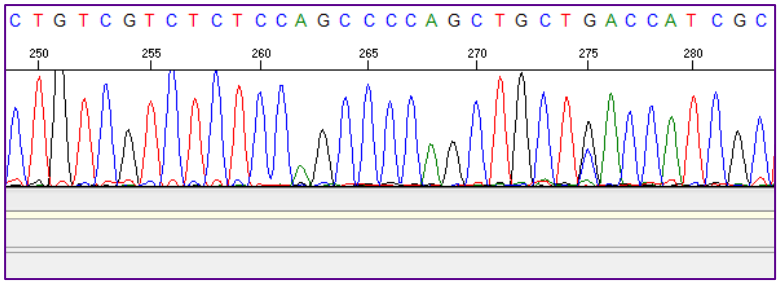


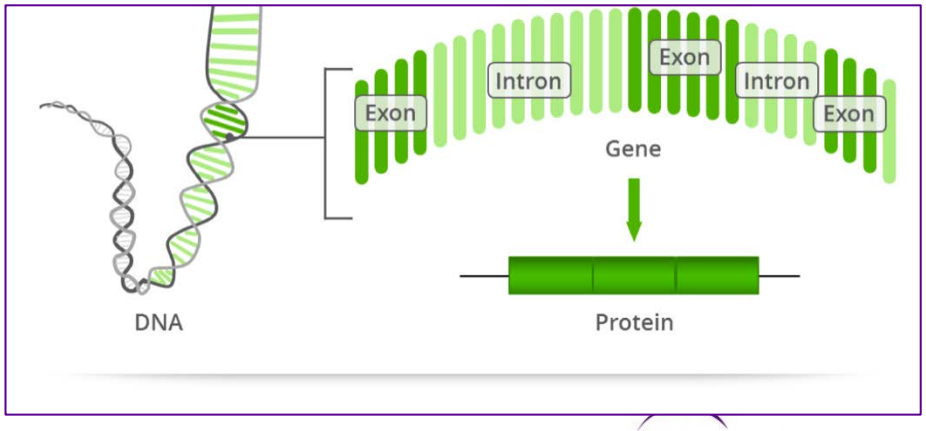
Somatic variant found
in tumor but not normal



Types of sequencing

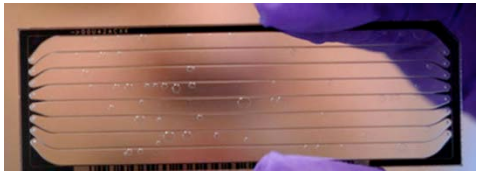
- Sanger sequencing (first generation) -2x coverage
- Next generation sequencing (second generation) – 30x to 1,000x coverage
  - Targeted sequencing
  - Hotspots (recurrent mutations at specific loci)
  - Exome sequencing (WES), 1-2% of the genome $$
  - Genome sequence (WGS) $$$$$

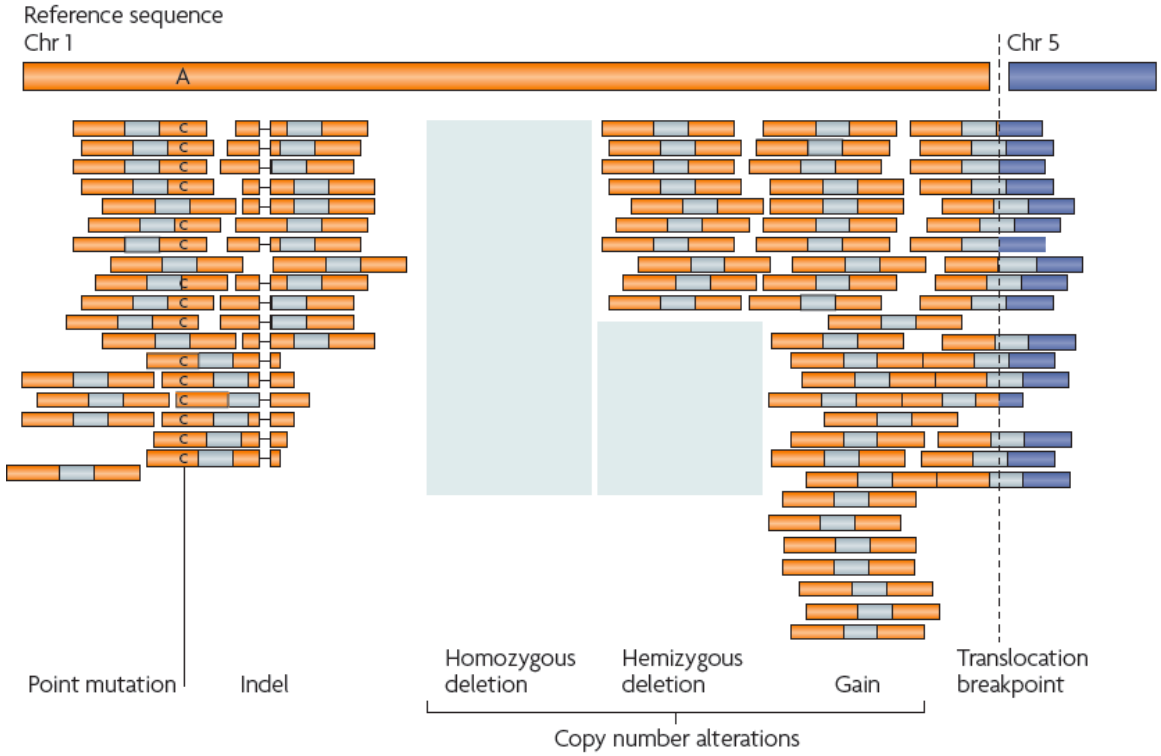




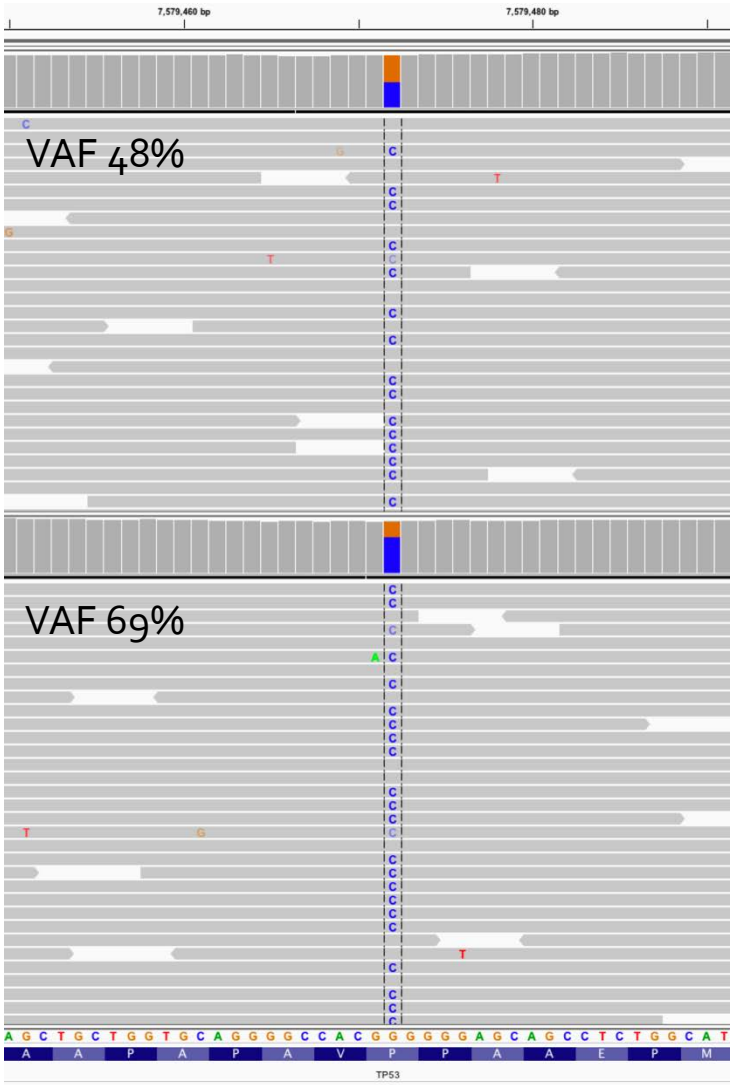
Next generation sequencing (NGS) is powerful

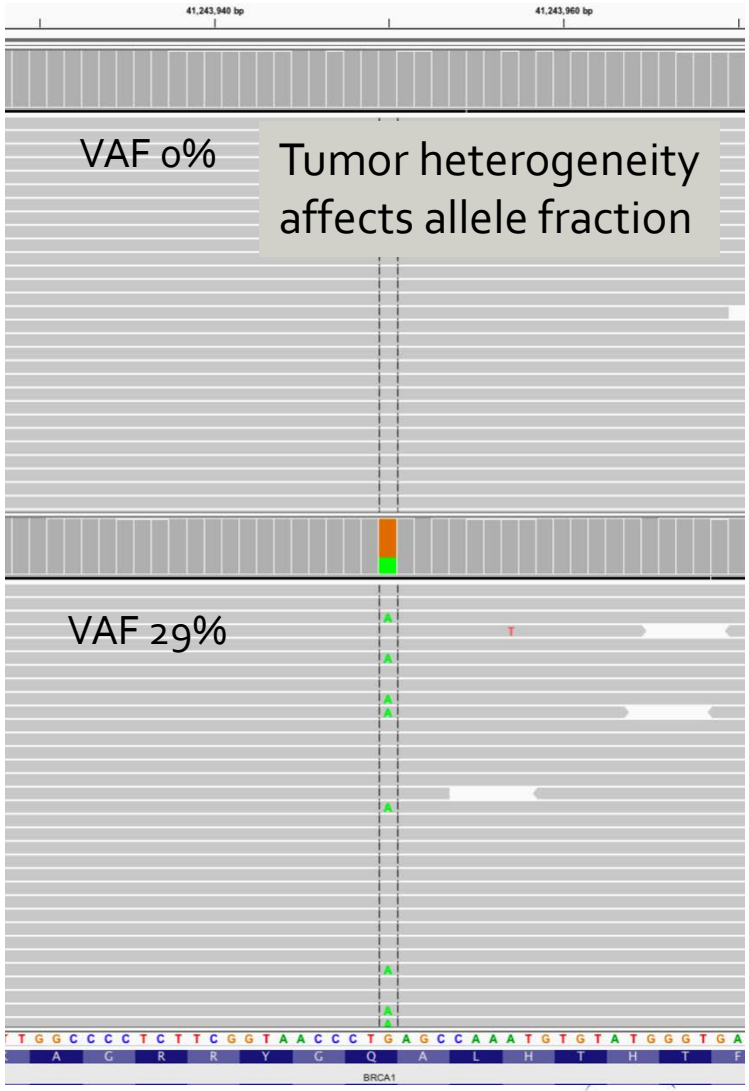
- NGS provides much more coverage and information than traditional Sanger Sequencing



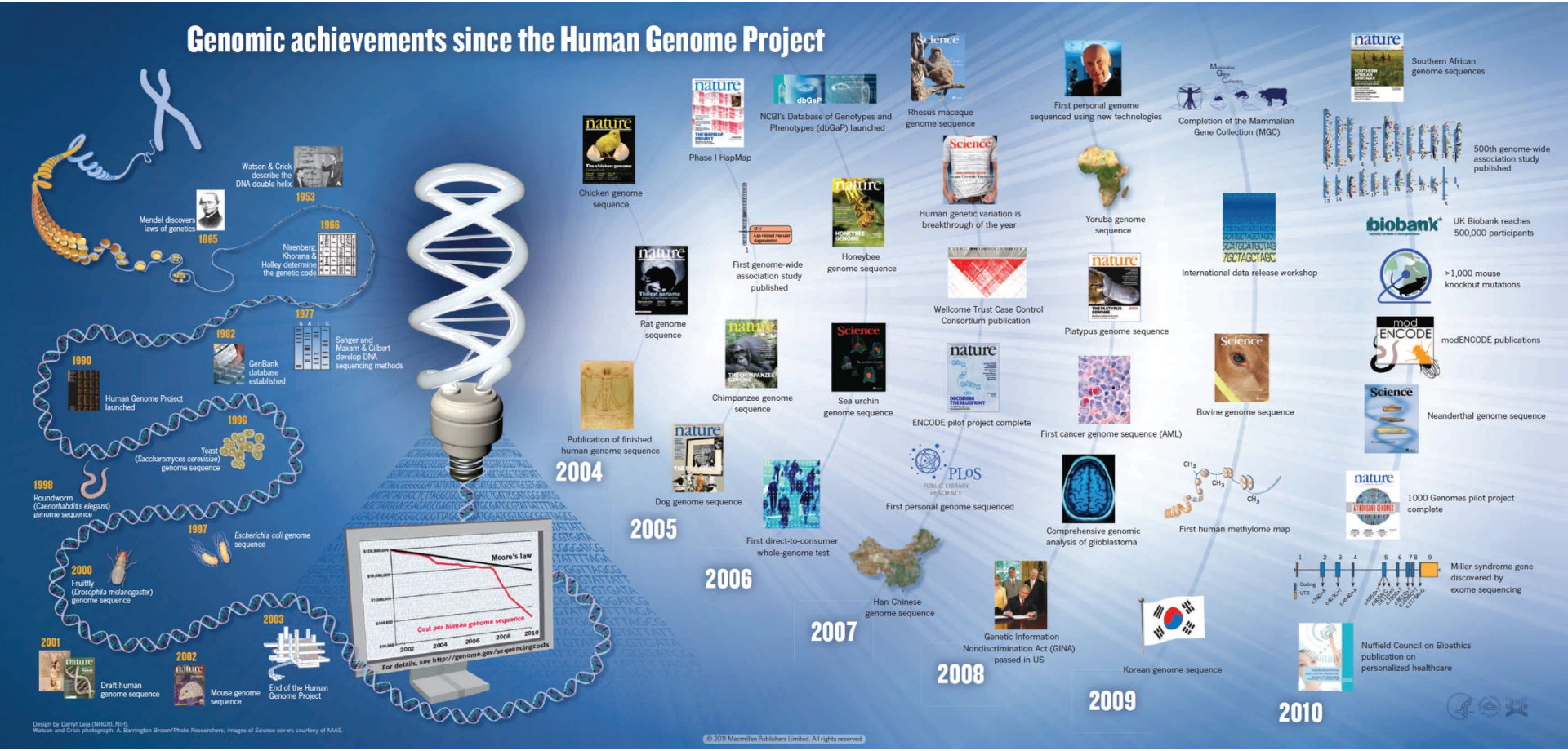


NGS germline and somatic examples





The human genome was the start of a molecular revolution



Genomics have defined the landscape of solid tumors

ARTICLE

OPEN

doi:10.1038/nature13385

Comprehensive molecular profiling of lung adenocarcinoma

The Cancer Genome Atlas Research Network*

doi:10.1038/nature10166

ARTICLE

Integrated genomic analyses of ovarian carcinoma

The Cancer Genome Atlas Research Network*

ARTICLE

OPEN

doi:10.1038/nature12113

Integrated genomic characterization of endometrial carcinoma

ARTICLES

The Cancer Genome Atlas Research Network*

ARTICLE

doi:10.1038/nature11404

Comprehensive genomic characterization of squamous cell lung cancers

The Cancer Genome Atlas Research Network*

ARTICLE

Cancer Cell

Article

CellPress

Integrated Molecular Characterization of Uterine Carcinosarcoma

Andrew D. Cherniack,[1,11] Hui Shen,[2,11] Vonn Walter,[3,11] Chip Stewart,[1,11] Bradley A. Murray,[1] Reanne Bowlby,[4] Xin Hu,[5] Shiyun Ling,[5] Robert A. Soslow,[6] Russell R. Broaddus,[5] Rosemary E. Zuna,[7] Gordon Robertson,[4] Peter W. Laird,[2] Raju Kucherlapati,[8] Gordon B. Mills,[5] The Cancer Genome Atlas Research Network, John N. Weinstein,[5] Jiashan Zhang,[9] Rehan Akbani,[5,*] and Douglas A. Levine[10,12,13,*]

ARTICLE

Comprehensive molec... of urothelial bladder

The Cancer Genome Atlas Research Network*

Comprehensive genomic characterization ...man glioblastoma genes and ...ways

...esearch Network*

doi:10.1038/nature11252

...molecular characterization ...and rectal cancer

Comprehensive molecular characterization of clear cell renal cell carcinoma

The Cancer Genome Atlas Research Network*

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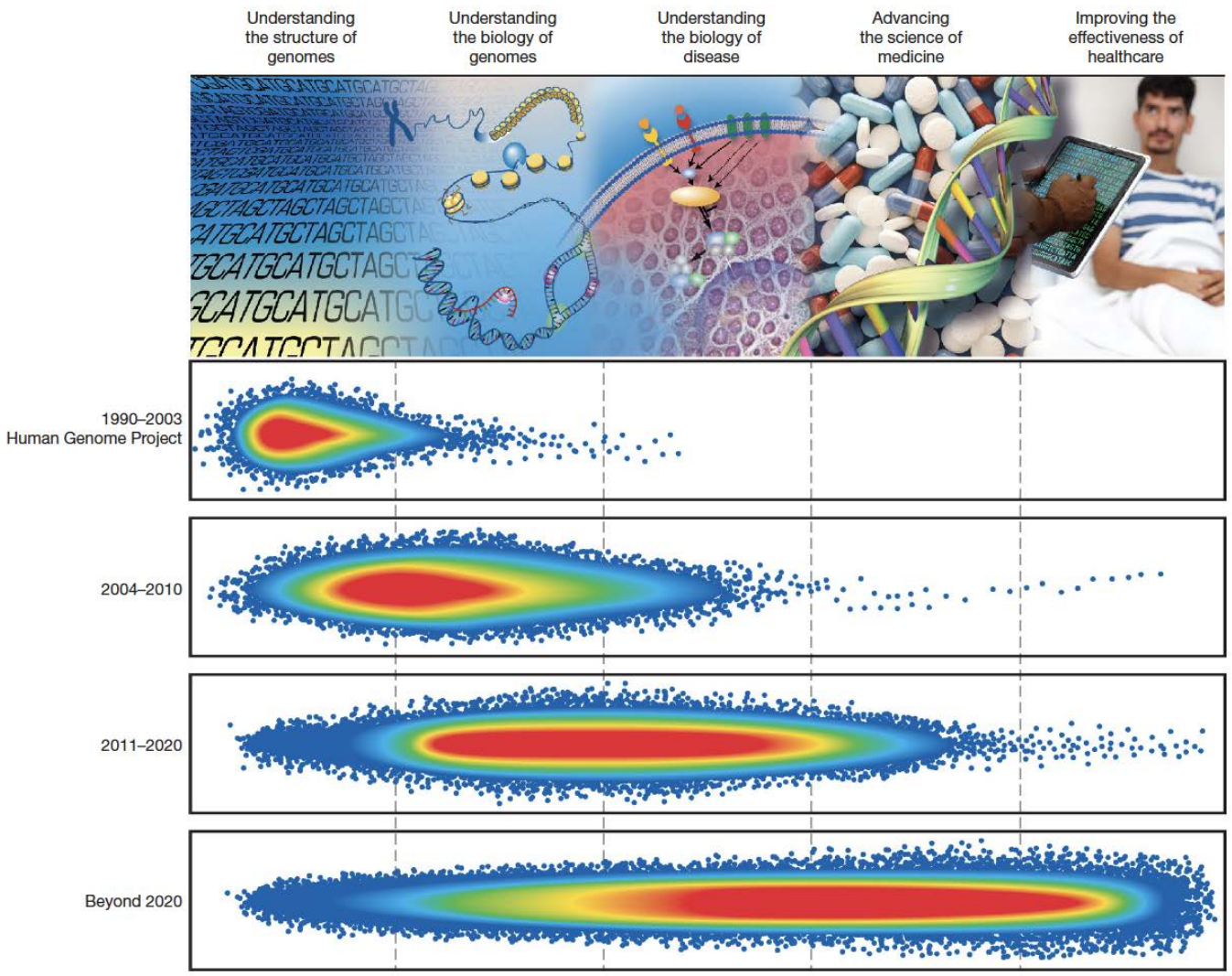
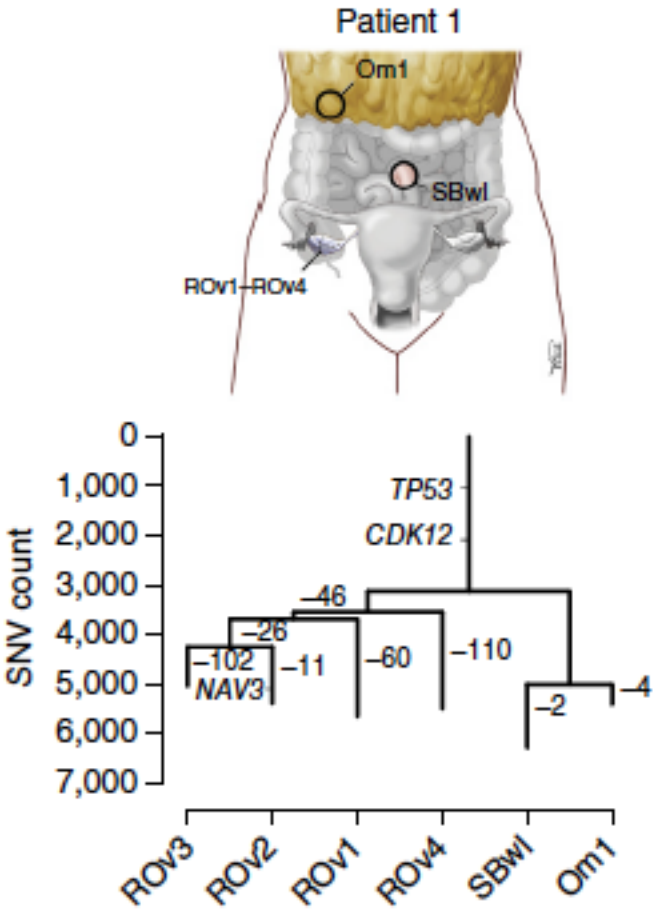
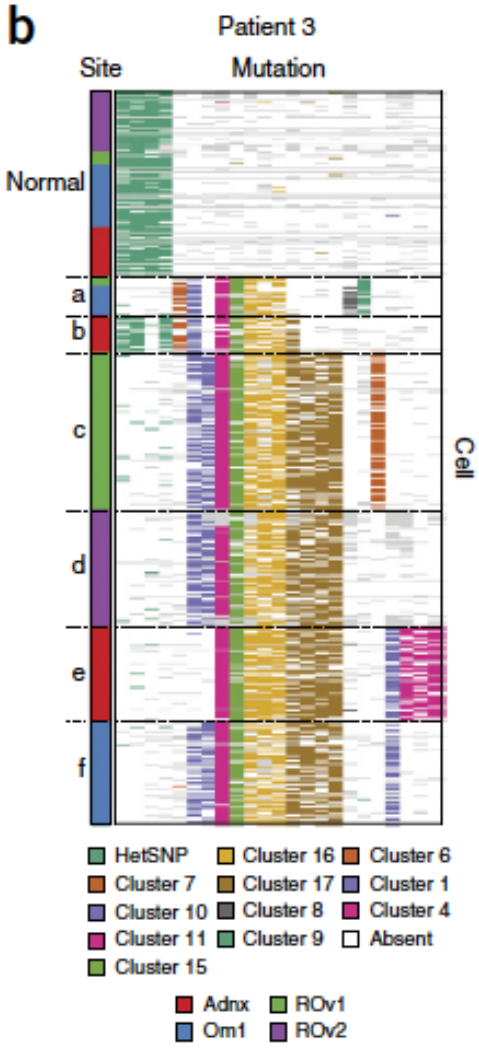
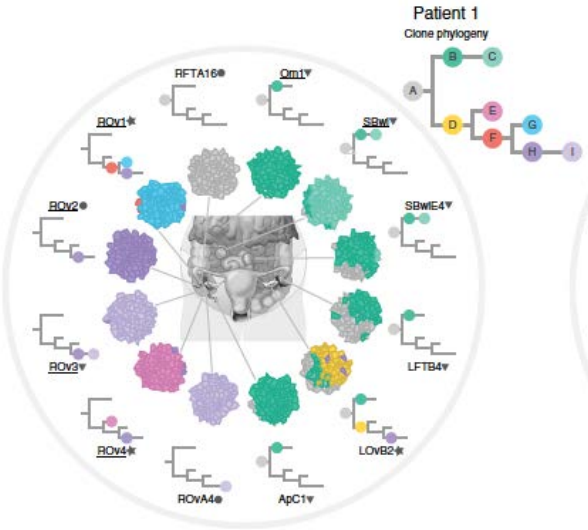
Precision medicine will move from discovery to therapy



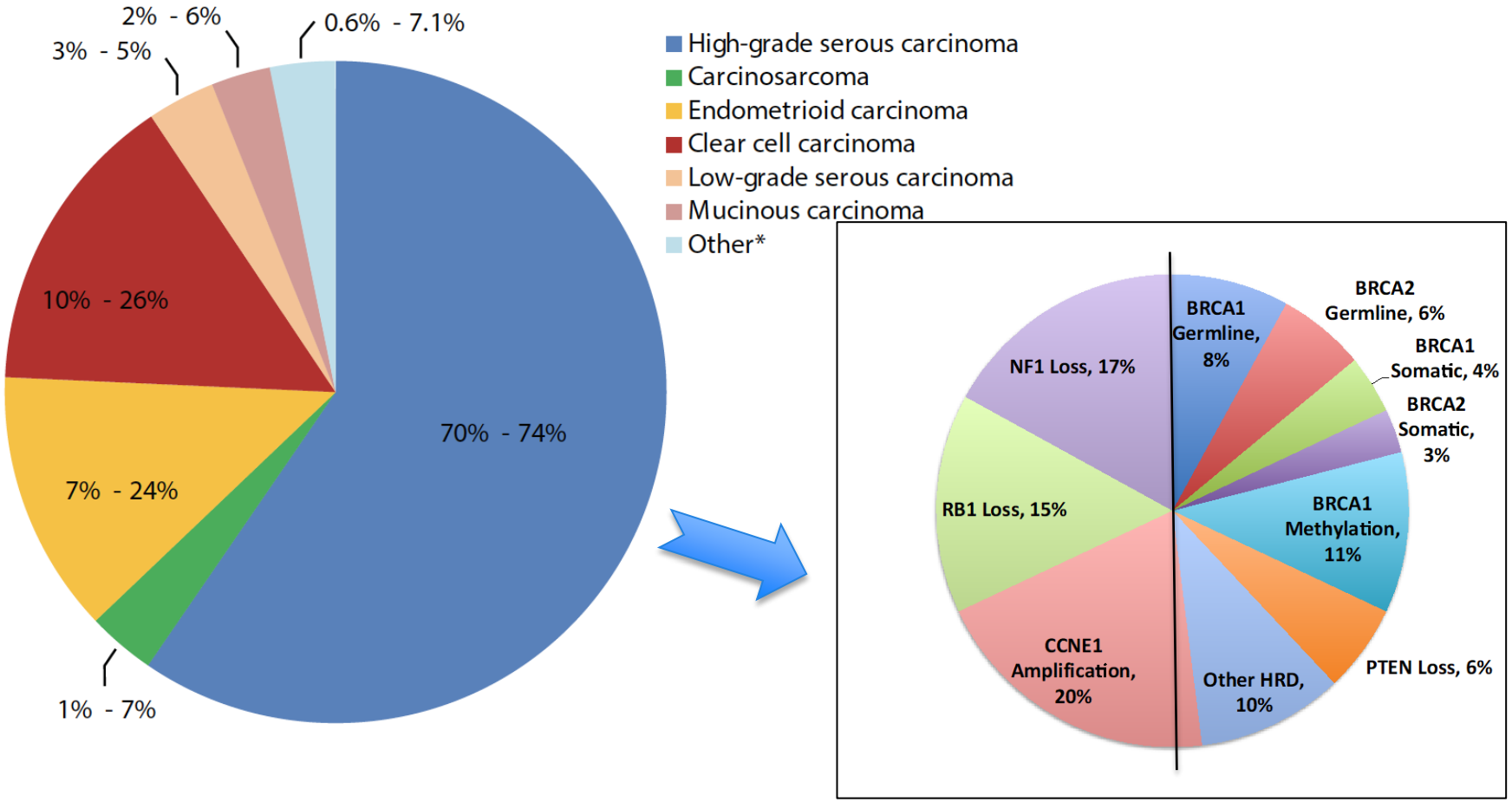
NGS can determine clonality and phylogeny







Ovarian carcinoma is not one disease.



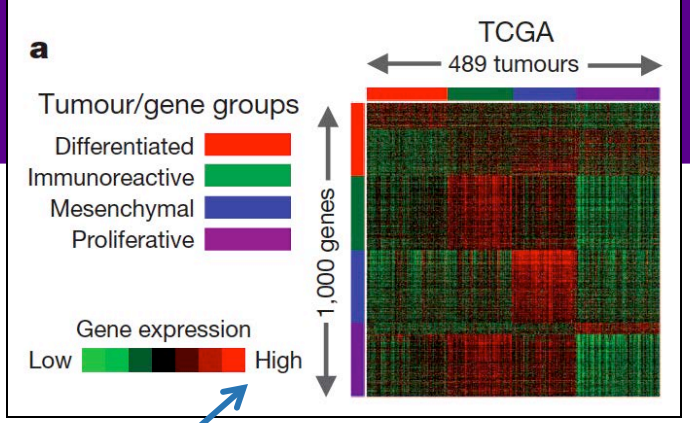
National Academies of Sciences, Engineering, and Medicine. 2016. Ovarian cancers: Evolving paradigms in research and care.

Created by Douglas A. Levine, MD from data compiled from the cBio Cancer Genomics Portal, MSKCC and Patch et al., Nature, 2015.

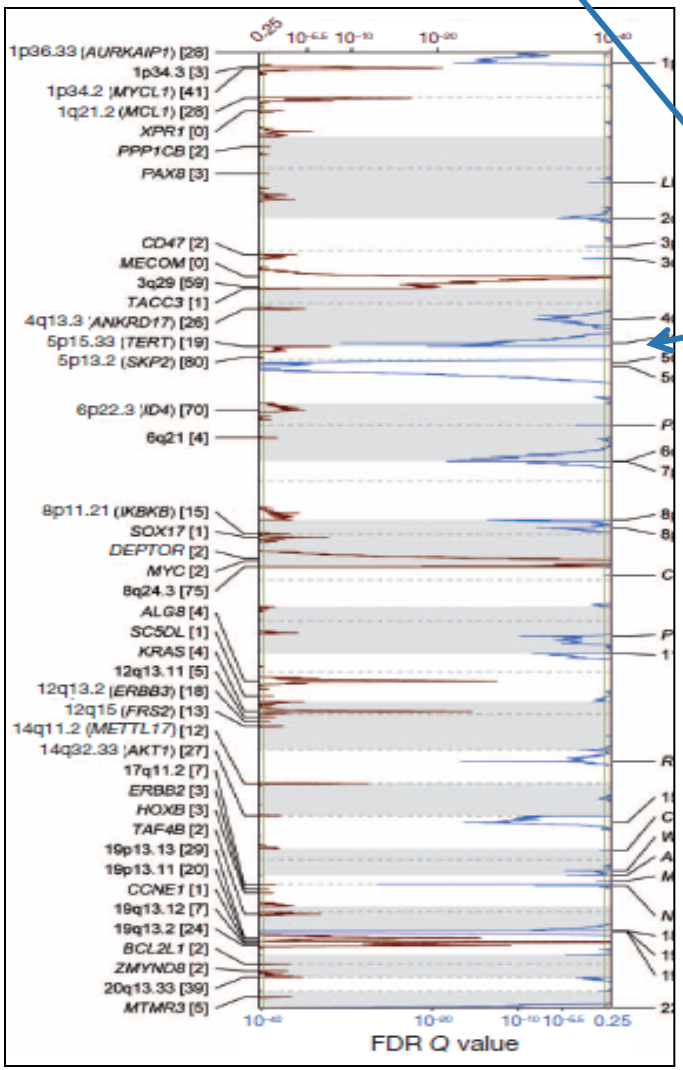
Table 2 | Significantly mutated genes in HGS-OvCa

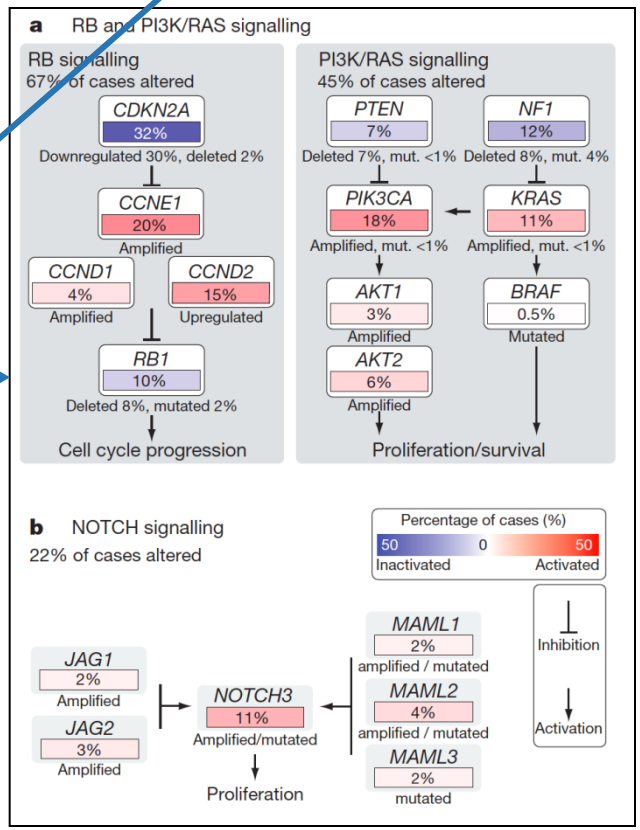
Gene	No. of mutations	No. validated	No. unvalidated
TP53	302	294	8
BRCA1	11	10	1
CSMD3	19	19	0
NF1	13	13	0
CDK12	9	9	0
FAT3	19	18	1
GABRA6	6	6	0
BRCA2	10	10	0
RB1	6	6	0

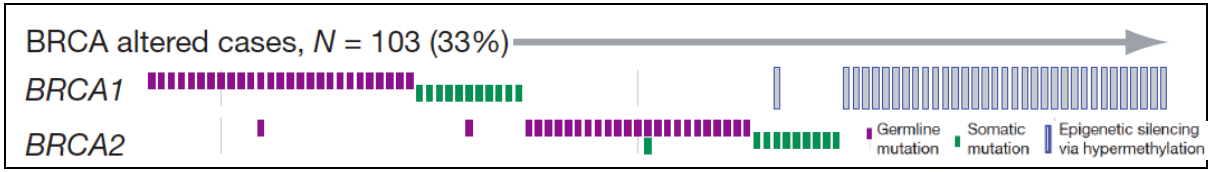
TCGA Findings- Ovarian Cancer



- Few recurrently mutated genes
- Four transcript subtypes
- Extensive copy number alterations
- Active canonical pathways
- Extensive defects homologous recombination with mutually exclusivity





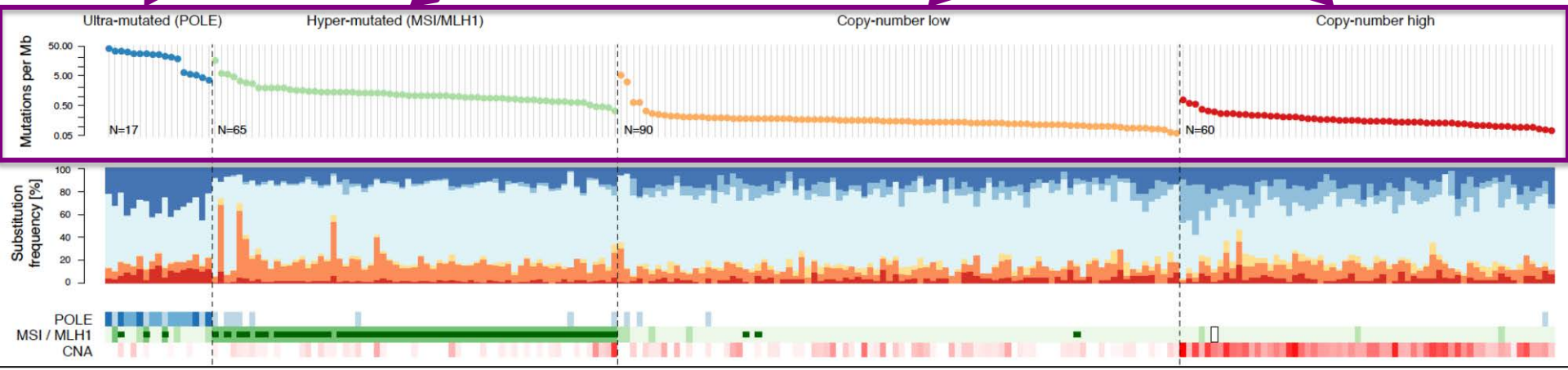


Mutation spectrum/subtypes of endometrial cancer

Very high background mutation rate; ~100-500 / Mb

High background mutation rate; ~10-20 / Mb

Low background mutation rate; ~2-3 / Mb



Hypermutators associated with MSI and MLH1 DNA promoter methylation

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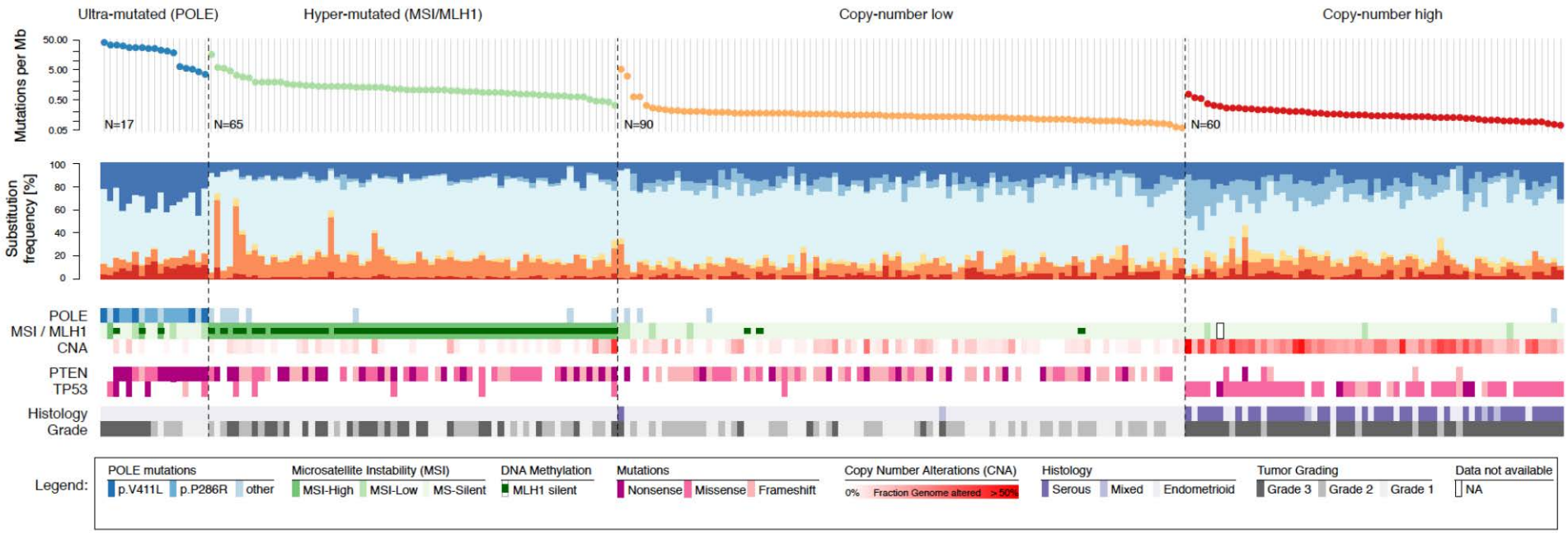
Integrated features



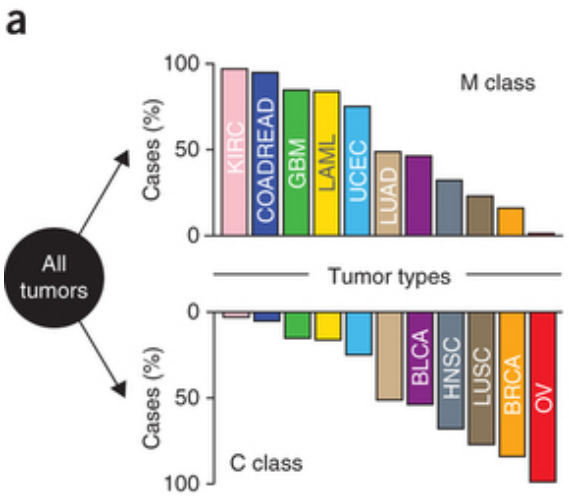
All endometrioid, PTEN mutations, few TP53 mutations

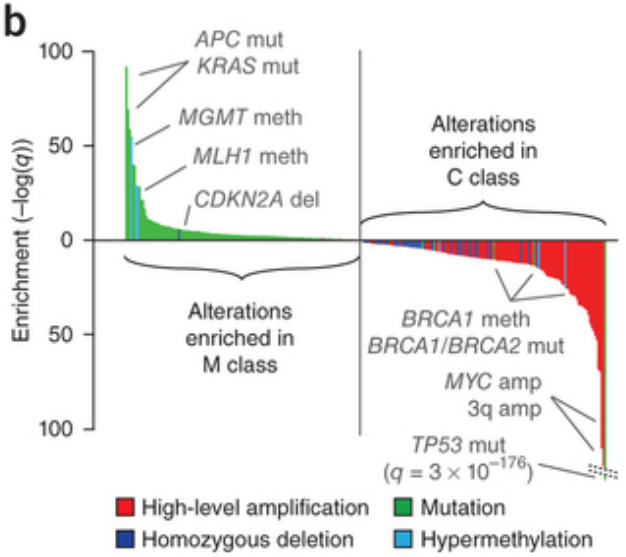
All endometrioid, PTEN mutations, no TP53 mutations

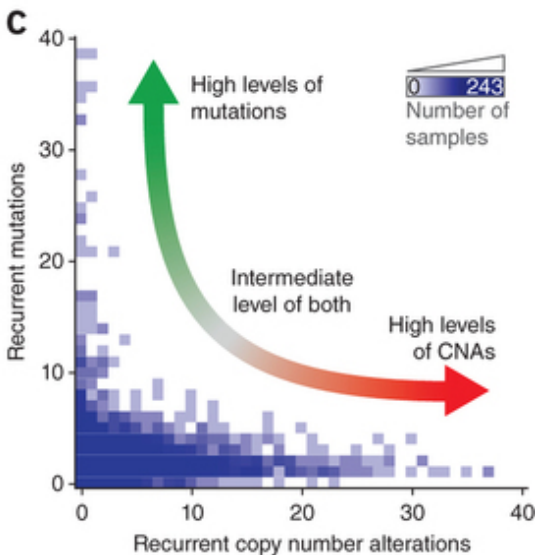
TP53 mutations, few PTEN mutations, high grade tumors, serous and some endometrioid

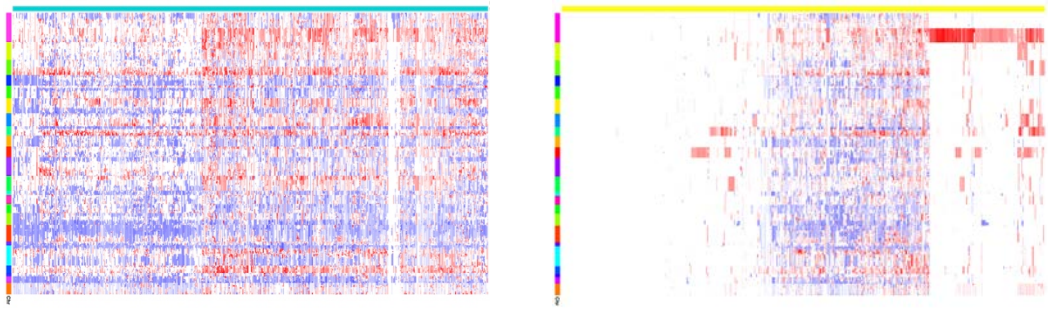
Emerging landscape of oncogenic signatures across human cancers

Giovanni Ciriello, Martin L Miller, Bülent Arman Aksoy, Yasin Senbabaoglu, Nikolaus Schultz & Chris Sander









Kitchen sink approach

Multiple tumor profiling technologies to decode cancer. This technology agnostic, panomic approach allows our medial teams to more fully understand the biology of the tumor – therefore helping oncologists' better plan their attack.

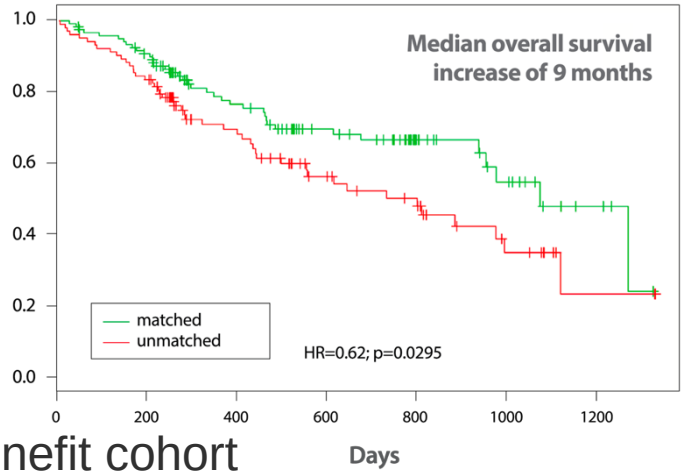
- **Immunohistochemistry (IHC):** determines level of protein expression
- ***in situ* Hybridization:** detects gene deletions, amplifications, translocations and fusions
- **Next-Generation Sequencing (NGS):** rapidly examines and more broadly detects DNA mutations, copy number variations and gene fusions across the genome
- **Sanger Sequencing:** examines strands of DNA to identify mutations by analyzing long contiguous sequencing reads
- **Pyro Sequencing (PyroSeq):** detects and quantifies mutations, methylation, etc. through sequencing by synthesis
- **Fragment Analysis (FA/Frag. Analysis):** detects changes in DNA or RNA to indicate the presence or absence of genetic marker

Kitchen sink approach

CARIS MOLECULAR INTELLIGENCE®

The Caris Molecular Intelligence® CGP+ approach to assess DNA, RNA and Proteins reveals the highest quality molecular blueprint to guide more precise and individualized treatment decisions that is proven to extend overall survival. As the first and most experienced tumor profiling service, we provide results back in about 8-10 days and to help you find better answers when you need it most.

Of 450 patients, a total of 278 were divided between two cohorts: the Benefit cohort included patients whose treatments were predicted to be of benefit (170) while the Lack of Benefit cohort included patients who received at least one treatment predicted to be of lack of benefit (178).



Median OS observed for patients included in the Benefit cohort (158.0 months) was significantly longer compared to patients included in the Lack of Benefit cohort (63.4 months) – see image below

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https://www.carismolecularintelligence.com/clinical-utility-tumor-profiling/

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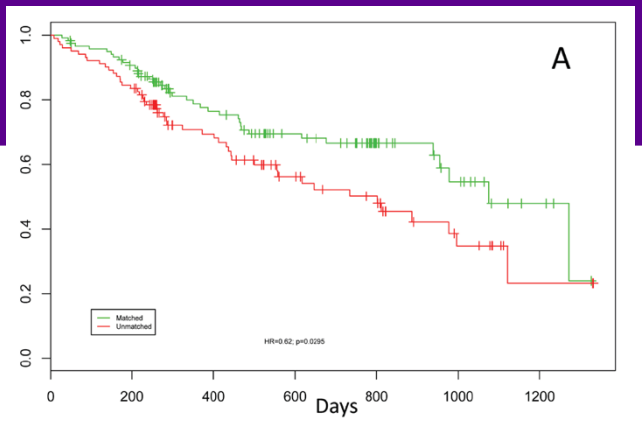
Let's read the paper…..

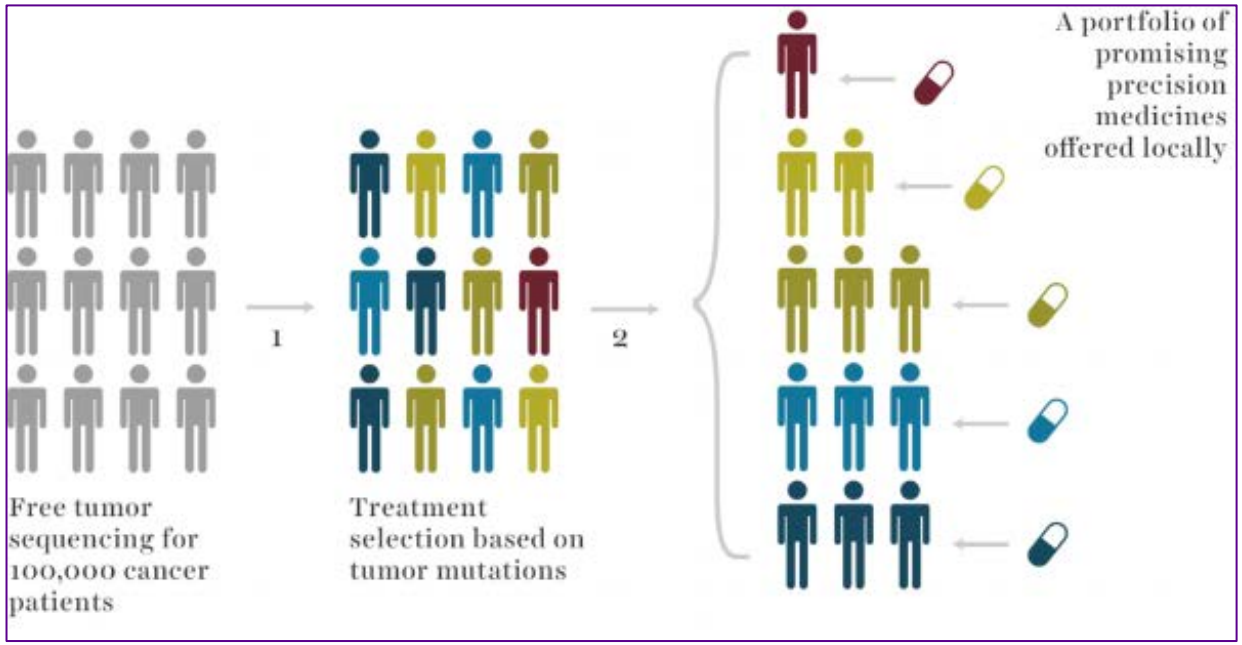


Patients in the Matched cohort experienced a significantly greater improvement in OS from the time of molecular profiling when compared to patients in the Unmatched cohort. Median OS from the time of tumor profiling for patients included in the Matched cohort was 36 months compared to 27 months for patients in the Unmatched cohort (HR 0.62, 95% CI 0.41-0.96; $p < 0.03$; Figure 4A). Patients who received more than

Oncotarget. 2016; 7:19840-19849. doi: 10.18632/oncotarget.7835

Caris: Median OS observed for patients included in the Benefit cohort (158.0 months) was significantly longer compared to patients included in the Lack of Benefit cohort (63.4 months) – see image below

STRATA ONCOLOGY



- 90-gene targeted sequencing assay focused on actionable genomic alterations: mutations, copy number, fusions, gene expression
- Sequences DNA and RNA from limited FFPE tumor material
- Less than 10 day turn-around time

PATIENT RESULTS

- 9 genomic findings
- 7 therapies associated with potential clinical benefit
- 0 therapies associated with lack of response
- 4 clinical trials

TUMOR TYPE: UTERUS CARCINOSARCOMA

Genomic Alterations Identified†

EGFR R831C
FOXP1 amplification
MED12 G44D – subclonal#
NOTCH1 Y1251fs*1
NOTCH3 BRD4-NOTCH3 rearrangement
SPTA1 R1077H
ZNF217 amplification – equivocal#

Additional Findings†

Microsatellite status MS-Stable
Tumor Mutation Burden TMB-Low; 4 Muts/Mb

† For a complete list of the genes assayed and performance specifications, please refer to the Appendix
# See Appendix for details

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VARIANTS OF UNKNOWN SIGNIFICANCE

Note: One or more variants of unknown significance (VUS) were detected in this patient's tumor. These variants may not have been adequately characterized in the scientific literature at the time this report was issued, and/or the genomic context of these alterations makes their significance unclear. We choose to include them here in the event that they become clinically meaningful in the future.

FAT1	IRS2	LRP1B	MED12
A467S	G1263V	Y3617H	A502V

THERAPEUTIC IMPLICATIONS

Genomic Findings Detected	FDA-Approved Therapies (in patient's tumor type)	FDA-Approved Therapies (in another tumor type)	Potential Clinical Trials
EGFR R831C	None	Cetuximab Erlotinib Gefitinib Lapatinib Panitumumab Afatinib Osimertinib	Yes, see clinical trials section
FOXP1 amplification	None	None	None
MED12 G44D - subclonal	None	None	None

GENOMIC ALTERATIONS

GENE ALTERATION	INTERPRETATION
◆ ***AKT2*** amplification - equivocal	**Gene and Alteration:** AKT2 encodes an intracellular serine/threonine kinase that is also known as PKB-beta. AKT2 is one of three members of the AKT gene family, and activation of AKT2 has been implicated in multiple malignancies[1,2]. AKT isoforms appear to have different roles in tumorigenesis; AKT1 appears to contribute to tumor initiation, whereas AKT2 promotes invasion and metastasis in breast tumors[3]. Although AKT2 amplification has been reported to associate with AKT2 overexpression[4,5,6], studies in various cancers suggest that AKT2 phosphorylation may have greater clinical relevance than AKT2 amplification or mRNA overexpression[7,8]. **Frequency and Prognosis:** Amplification of AKT2 has been found in 6% of ovarian serous cystadenocarcinoma cases[9]. AKT2 amplification has been reported frequently in ovarian carcinoma tumors and cell lines[4,5,10]. **Potential Treatment Strategies:** Amplification of AKT2 may promote PI3K-AKT-mTOR pathway activation and may predict sensitivity to inhibitors of the AKT and downstream mTOR pathways. AKT inhibitors are in clinical trials in various tumor types. The mTOR inhibitors everolimus and temsirolimus have been FDA approved in other indications, and these agents as well as other mTOR inhibitors, alone and in combination with other therapies, are also in clinical trials in multiple solid tumor types. In preclinical studies, the AKT inhibitor MK-2206 has been shown to enhance antitumor activity of other chemotherapies in serous ovarian carcinoma and other tumor cells[11,12].

APPENDIX

GENES ASSAYED IN FOUNDATIONONE

FoundationOne is designed to include all genes known to be somatically altered in human solid tumors that are validated targets for therapy, either approved or in clinical trials, and/or that are unambiguous drivers of oncogenesis based on current knowledge. The current assay interrogates 315 genes as well as introns of 28 genes involved in rearrangements. The assay will be updated periodically to reflect new knowledge about cancer biology.

DNA Gene List: Entire Coding Sequence for the Detection of Base Substitutions, Insertion/Deletions, and Copy Number Alterations

ABL1	ABL2	ACVR1B	AKT1	AKT2	AKT3	ALK	AMER1 (FAM123B)	APC	AR
ARAF	ARFRP1	ARID1A	ARID1B	ARID2	ASXL1	ATM	ATR	ATRX	AURKA
AURKB	AXIN1	AXL	BAP1	BARD1	BCL2	BCL2L1	BCL2L2	BCL6	BCOR
BCORL1	BLM	BRAF	BRCA1	BRCA2	BRD4	BRIP1	BTG1	BTK	C11orf30 (EMSY)
CARD11	CBFB	CBL	CCND1	CCND2	CCND3	CCNE1	CD274	CD79A	CD79B
CDC73	CDH1	CDK12	CDK4	CDK6	CDK8	CDKN1A	CDKN1B	CDKN2A	CDKN2B
CDKN2C	CEBPA	CHD2	CHD4	CHEK1	CHEK2	CIC	CREBBP	CRKL	CRLF2
CSF1R	CTCF	CTNNA1	CTNNB1	CUL3	CYLD	DAXX	DDR2	DICER1	DNMT3A
DOT1L	EGFR	EP300	EPHA3	EPHA5	EPHA7	EPHB1	ERBB2	ERBB3	ERBB4
ERG	ERRFI1	ESR1	EZH2	FAM46C	FANCA	FANCC	FANCD2	FANCE	FANCF
FANCG	FANCL	FAS	FAT1	FBXW7	FGF10	FGF14	FGF19	FGF23	FGF3
FGF4	FGF6	FGFR1	FGFR2	FGFR3	FGFR4	FH	FLCN	FLT1	FLT3
FLT4	FOXL2	FOXP1	FRS2	FUBP1	GABRA6	GATA1	GATA2	GATA3	GATA4
GATA6	GID4 (C17orf39)	GLI1	GNA11	GNA13	GNAQ	GNAS	GPR124	GRIN2A	GRM3
GSK3B	H3F3A	HGF	HNF1A	HRAS	HSD3B1	HSP90AA1	IDH1	IDH2	IGF1R
IGF2	IKBKE	IKZF1	IL7R	INHBA	INPP4B	IRF2	IRF4	IRS2	JAK1
JAK2	JAK3	JUN	KAT6A (MYST3)	KDM5A	KDM5C	KDM6A	KDR	KEAP1	KEL
KIT	KLHL6	KMT2A (MLL)	KMT2C (MLL3)	KMT2D (MLL2)	KRAS	LMO1	LRP1B	LYN	LZTR1
MAGI2	MAP2K1	MAP2K2	MAP2K4	MAP3K1	MCL1	MDM2	MDM4	MED12	MEF2B
MEN1	MET	MITF	MLH1	MPL	MRE11A	MSH2	MSH6	MTOR	MUTYH
MYC	MYCL (MYCL1)	MYCN	MYD88	NF1	NF2	NFE2L2	NFKBIA	NKX2-1	NOTCH1
NOTCH2	NOTCH3	NPM1	NRAS	NSD1	NTRK1	NTRK2	NTRK3	NUP93	PAK3
PALB2	PARK2	PAX5	PBRM1	PDCD1LG2	PDGFRA	PDGFRB	PDK1	PIK3C2B	PIK3CA
PIK3CB	PIK3CG	PIK3R1	PIK3R2	PLCG2	PMS2	POLD1	POLE	PPP2R1A	PRDM1
PREX2	PRKAR1A	PRKCI	PRKDC	PRSS8	PTCH1	PTEN	PTPN11	QKI	RAC1
RAD50	RAD51	RAF1	RANBP2	RARA	RB1	RBM10	RET	RICTOR	RNF43
ROS1	RPTOR	RUNX1	RUNX1T1	SDHA	SDHB	SDHC	SDHD	SETD2	SF3B1
SLIT2	SMAD2	SMAD3	SMAD4	SMARCA4	SMARCB1	SMO	SNCAIP	SOCS1	SOX10
SOX2	SOX9	SPEN	SPOP	SPTA1	SRC	STAG2	STAT3	STAT4	STK11
SUFU	SYK	TAF1	TBX3	TERC	TERT (promoter only)	TET2	TGFBR2	TNFAIP3	TNFRSF14
TOP1	TOP2A	TP53	TSC1	TSC2	TSHR	U2AF1	VEGFA	VHL	WISP3
WT1	XPO1	ZBTB2	ZNF217	ZNF703					

DNA Gene List: For the Detection of Select Rearrangements

ALK	BCL2	BCR	BRAF	BRCA1	BRCA2	BRD4	EGFR	ETV1	ETV4
ETV5	ETV6	FGFR1	FGFR2	FGFR3	KIT	MSH2	MYB	MYC	NOTCH2
NTRK1	NTRK2	PDGFRA	RAF1	RARA	RET	ROS1	TMPRSS2		

Additional Assays: For the Detection of Select Cancer Biomarkers

Microsatellite status
Tumor Mutation Burden

CLINICAL TRIALS TO CONSIDER

GENE	RATIONALE FOR POTENTIAL CLINICAL TRIALS
● ***TP53*** I255S	TP53 loss of function alterations may predict sensitivity to WEE1 inhibitors or therapies that reactivate mutant p53. Examples of clinical trials that may be appropriate for this patient are listed below. These trials were identified through a search of the trial website clinicaltrials.gov using keyword terms such as "p53", "AZD1775", "MK1775", "WEE1", "ALT-801", "p53MVA", "APR-246", "ovarian carcinoma", "solid tumor", and/or "advanced cancers".

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NGS can assist with clinical trial recruitment

- 52yo with BRCA negative ovarian cancer who recurred six months after complete resection
- She had a biopsy of her recurrent liver lesion
- She was recommended to enroll on a randomized phase II of cabozantinib vs paclitaxel, but did not like randomization options (only wanted targeted therapy)
- Repeat testing after 4 years suggests similar targets with enrichment

TUMOR TYPE: OVARY SEROUS CARCINOMA

Genomic Alterations Identified† 2012

AKT3 amplification
MET amplification
NF1 deletion, exon 1
MYC amplification
MYCL1 amplification
TP53 A84fs*39
BRAF rearrangement, complex, intron 9
FGF6 V139I

†For a complete list of the genes assayed, please refer to the Appendix

TUMOR TYPE: OVARY EPITHELIAL CARCINOMA (NOS)

Genomic Alterations Identified† 2016

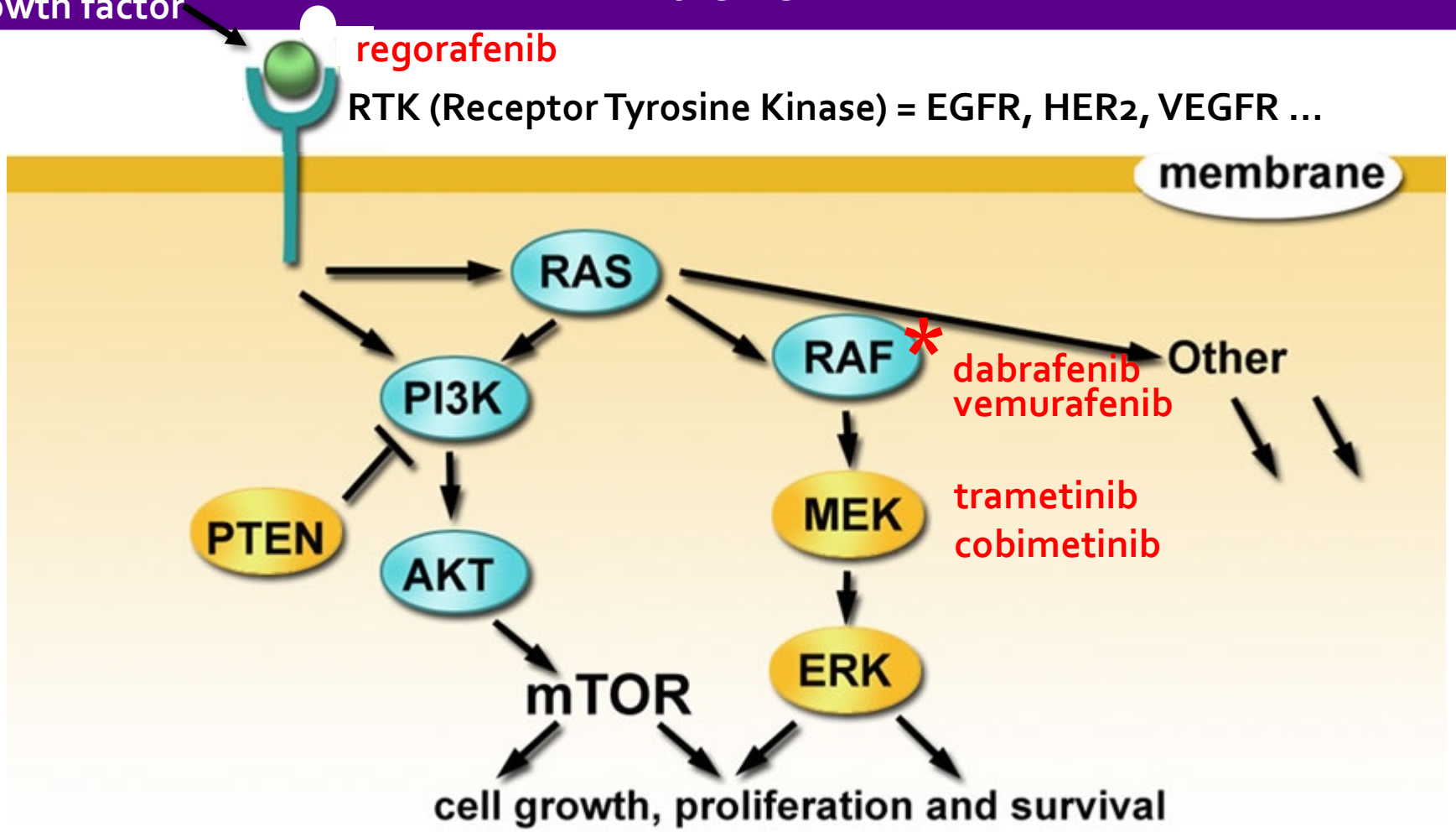
AKT3 amplification – equivocal#
BRAF duplication exons 10-18
MET amplification – equivocal#
NF1 SPATA13-NF1 rearrangement
TP53 A84fs*39
CCND3 amplification – equivocal#
FGF6 V139I

Additional Disease-relevant Genes with No Reportable Alterations Identified†

BRCA1
BRCA2

† For a complete list of the genes assayed and performance specifications, please refer to the Appendix
# See Appendix for details

RAS/RAF/PI3K/Akt/mTOR pathway overview



The RAF-inhibitors **dabrafenib** and **vemurafenib**, alone and in combination with the MEK-inhibitors **trametinib** and **cobimetini**b respectively, are FDA-approved for the treatment of patients with BRAF V600E and V600K mutant melanoma. Data support using the combination therapies dabrafenib plus trametinib or vemurafenib plus cobimetinib instead of single agent dabrafenib, vemurafenib, or trametinib.

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Adapted from: Han CB et al. *Journal of Cancer Therapy*; 2011

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Many profiles are stable across time and space

- 59yo with recurrent uterine carcinosarcoma
- Offered EGFR antibody, but developed liver failure and chose best supportive care

2016 uterus

TUMOR TYPE: UTERUS CARCINOSARCOMA

Genomic Alterations Identified†

EGFR R831C
FOXP1 amplification
MED12 G44D – subclonal#
NOTCH1 Y1251fs*1
NOTCH3 BRD4-NOTCH3 rearrangement
SPTA1 R1077H
ZNF217 amplification – equivocal#

Additional Findings†

Microsatellite status MS-Stable
Tumor Mutation Burden TMB-Low; 4 Muts/Mb

2017 liver

TUMOR TYPE: UTERUS CARCINOSARCOMA

Genomic Alterations Identified†

EGFR R831C
MYC amplification – equivocal#
FOXP1 amplification
NOTCH1 Y1251fs*1
NOTCH3 BRD4-NOTCH3 rearrangement
SPTA1 R1077H

Additional Findings†

Microsatellite status MS-Stable
Tumor Mutation Burden TMB-Low; 4 Muts/Mb

Endometrial carcinoma is not one disease

- Endometrial cancer is not one disease

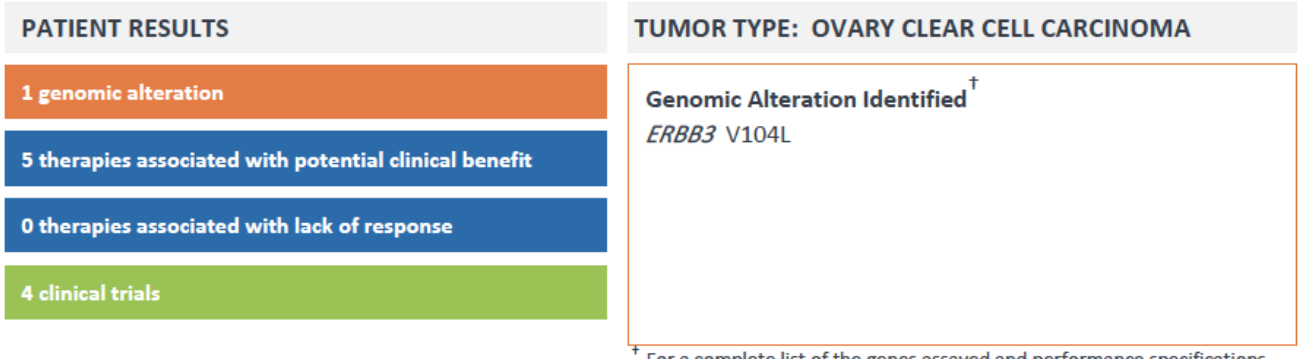
	Endometrioid	Serous	Carcinosarcoma	Clear cell
Bokhman subtype	I	II	II	II
TP53 mutation	Rare	>90%	60–90%	35%
PI3K alterations	*PTEN* mutation (75–85%) *PIK3CA* mutation (50–60%) *PIK3R1* mutation (40–50%)	*PTEN* mutation (11%) *PIK3CA* amplification (45%) *PIK3CA* mutation (35%) *PIK3R1* mutation (12%)	*PTEN* mutation (19%) *PIK3CA* mutation (35%) *PIK3CA* amplification (14%)	*PTEN* loss (80%) *PIK3CA* mutation (18%)
KRAS mutation	20–30%	3%	17%	0%
ERBB alterations	None	*ERBB2* amplification (25–30%)	*ERBB2* amplification (13–20%) *ERBB3* amplification or mutation (13%)	*ERBB2* mutation (12%) *ERBB2* amplification (16%)
FGFR amplification or mutation	*FGFR2* mutation (12%)	*FGFR2* mutation (5%) Frequent *FGFR1* and *FGFR3* amplification	*FGFR3* amplification (20%)	..
Wnt/β-catenin	*CTNNB1* mutation (25%)	*CTNNB1* mutation (3%)	..	..
Other	*ARID1A* mutation (35–40%)	*PPP2R1A* mutation (20%) *FBXW7* mutation (20% of undifferentiated endometrial carcinoma) *LRPB1* deletion Frequent amplifications in *MYC*, *CCNE1*, and *SOX17*	*PPP2R1A* mutation (28%) *FBXW7* mutation (35–40%) *ARID1A* mutation (25%) *CCNE1* amplification (42%) *SOX17* amplification (25%)	*ARID1A* (25%) *TERT* promoter mutations

Table 2: **Molecular classification of endometrial cancers, by histology**

Morice P et al. *Lancet.* 2016;387:1094-108.

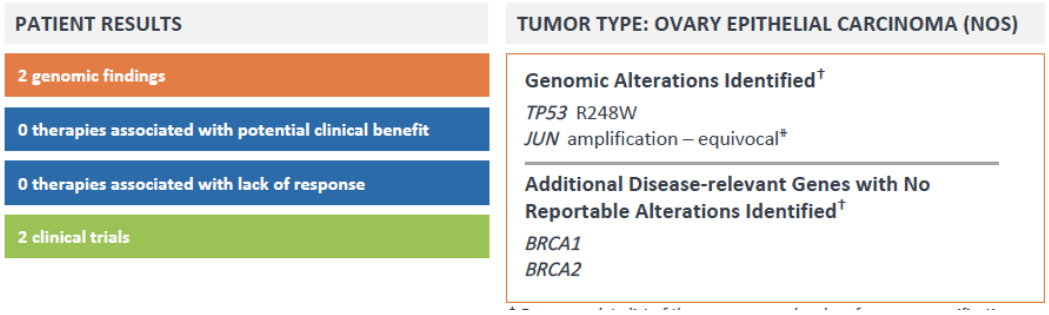
Rare cancer triaged to treatment with NGS

- 73yo with clear cell endometrial caner who progressed after taxol/ carbo had sequencing
- She was referred for neratinib trial, but did not qualify
- She was started on lapatinib, but progressed after one month of treatment

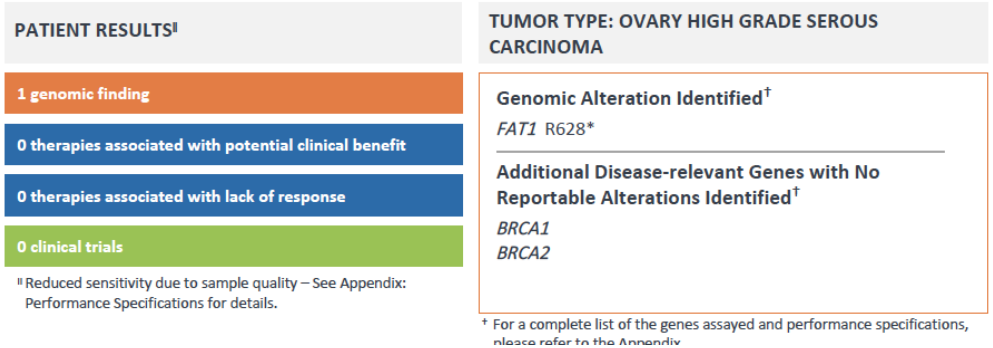


Results can be challenging

- 59 yo with HGSC and brain metastasis, biopsied



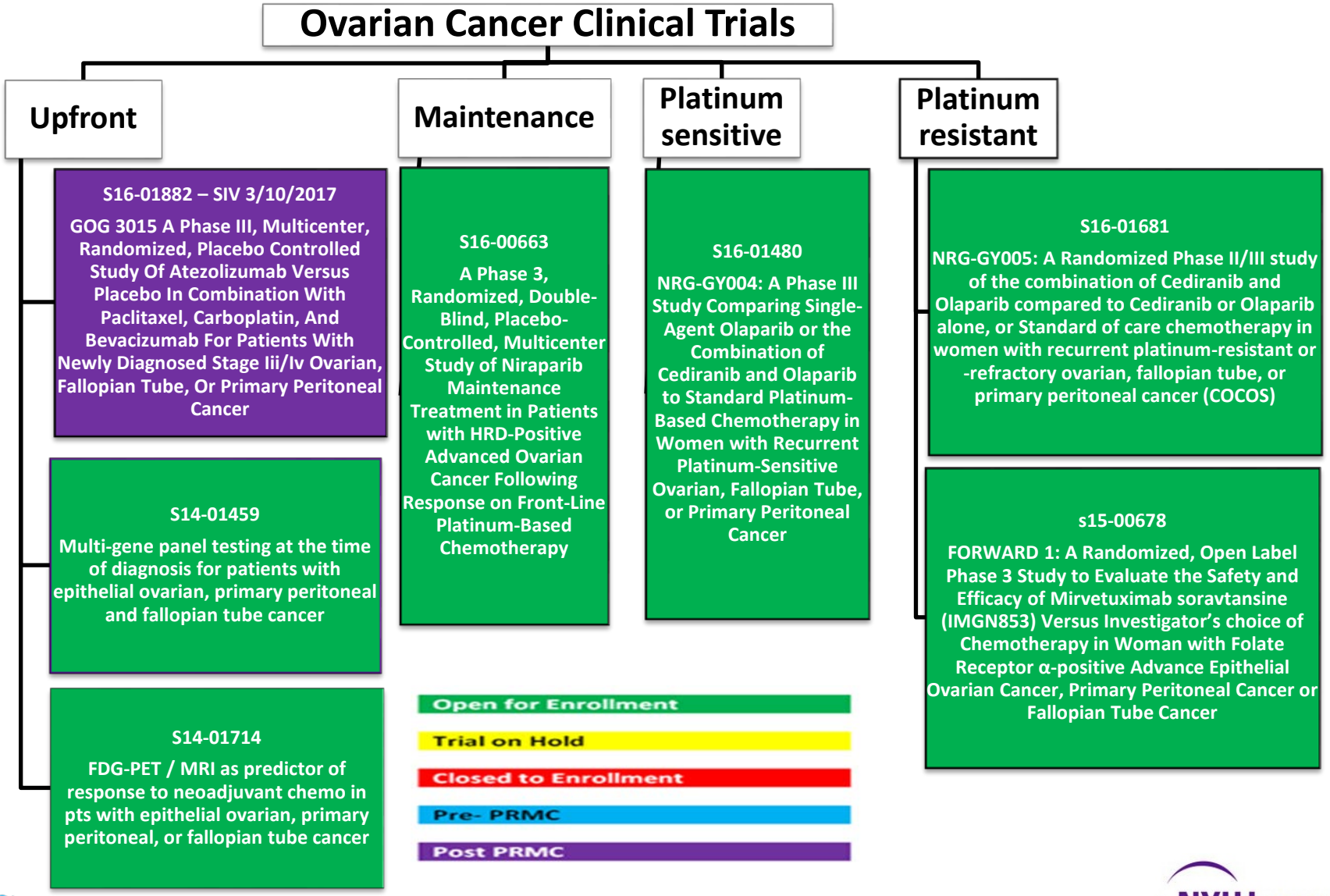
- 84yo with HGSC and IHC overexpression of TP53



Are you confused yet?

- Precision medicine is not the same as individualized medicine
  - Over-ambitious to have unique treatment for every patient
  - Anecdotal responses will lead to basket trials and treatment knowledge across histologic subtypes
- Genomic testing must be applied and interpreted in the appropriate contexts

Ovarian cancer clinical trials are available across the treatment continuum at Perlmutter Cancer Center.



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It takes a village to conquer gynecologic cancer.

Congressionally Directed Medical Research Programs
CDMRP
Department of Defense

NIH
National Cancer Institute

Tina's Wish

Katie Oppo Research Fund

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