Genomics and Personalized Cancer Treatment

David Huntsman

OvCaRe BC and The Centre for Translational and Applied Genomics

BC Cancer Agency, VGH , UBC

dhuntsma@bccancer.bc.ca

Basic Research and Health Care Needs

- There is still a great need for basic or fundamental research: in basic or fundamental research the specific applicability is unknown and thus researchers should not be obliged to declare a translational pathway
- The are many flavors of translational research: in all the health care needs must be defined and a translational pathway predetermined
- Our health care ‘system’ is built for the 1950’s and not well equipped for translation and transfer of knowledge

The British Columbia Personalized Medicine Initiative

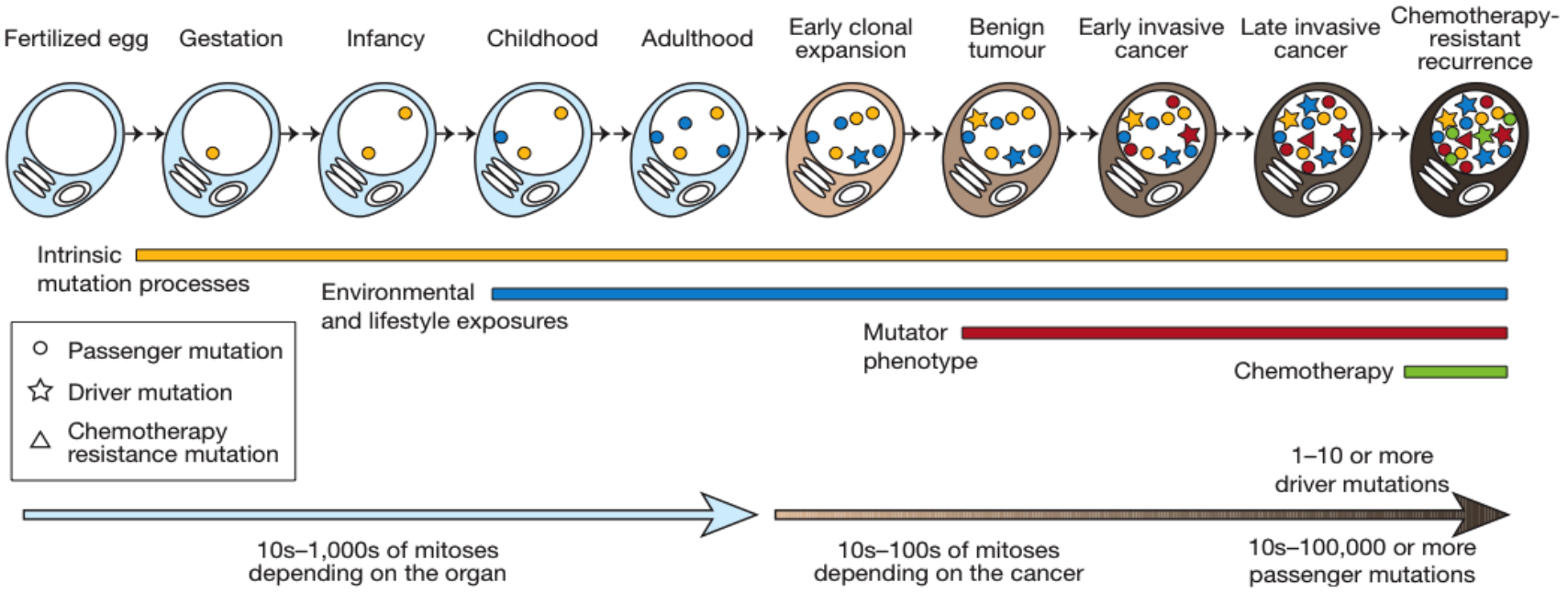
From individualized health solutions to better health for all

BC-PMI

One size does not fit all…

Operations Group: Pieter Cullis, David Huntsman, Michael Hayden, Bruce McManus, Michael Burgess, Jim Russell **Operations Officer:** Rob Fraser (robertfr@mail.ubc.ca)

Cancer as a genetic disease

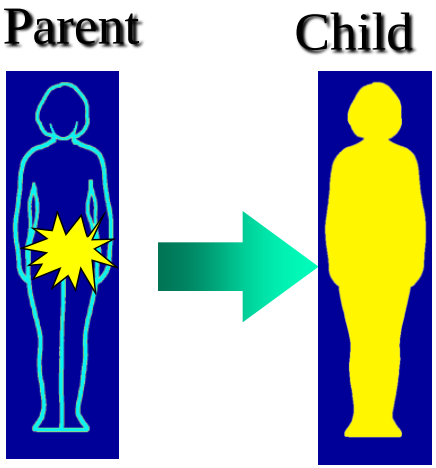


Stratton Nature, 2009

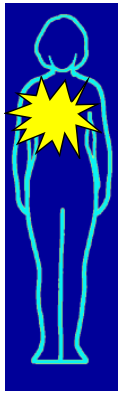
Cancer: A tale of two Genomes

Germline SNP's and mutations

Mutation in egg or sperm

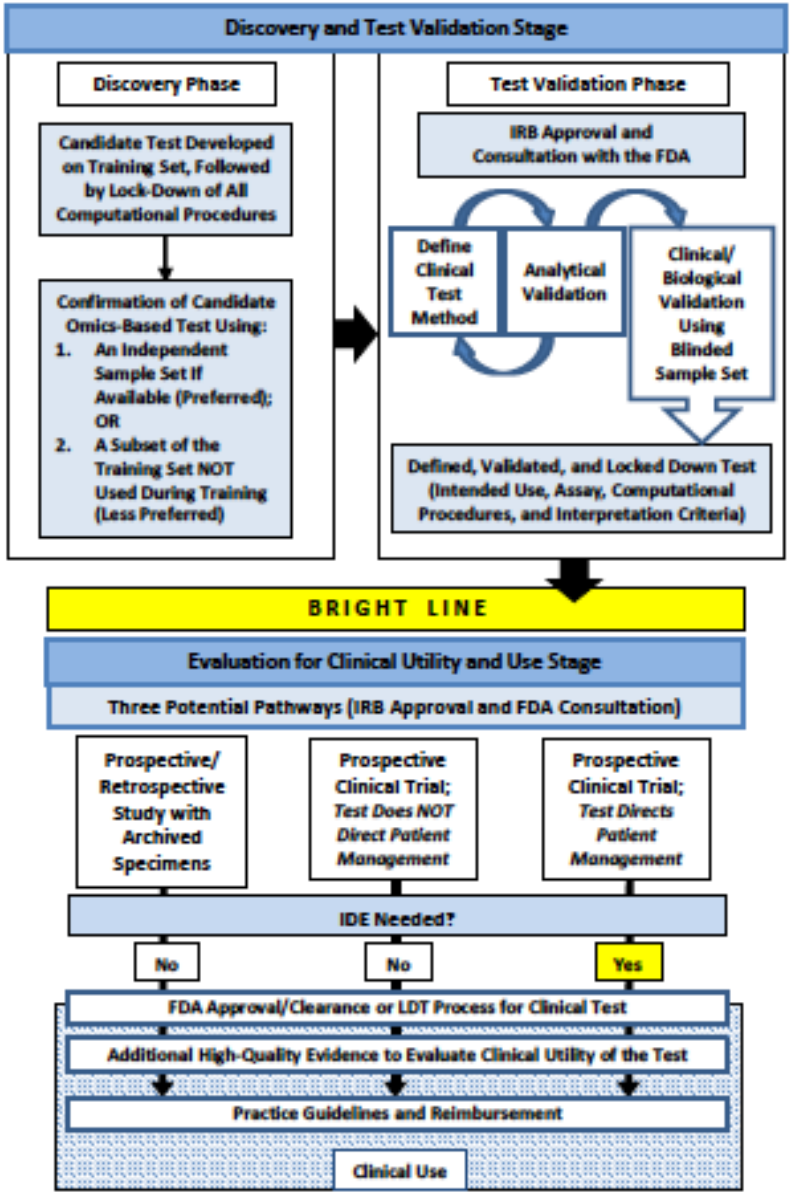


Somatic mutations



Somatic mutations

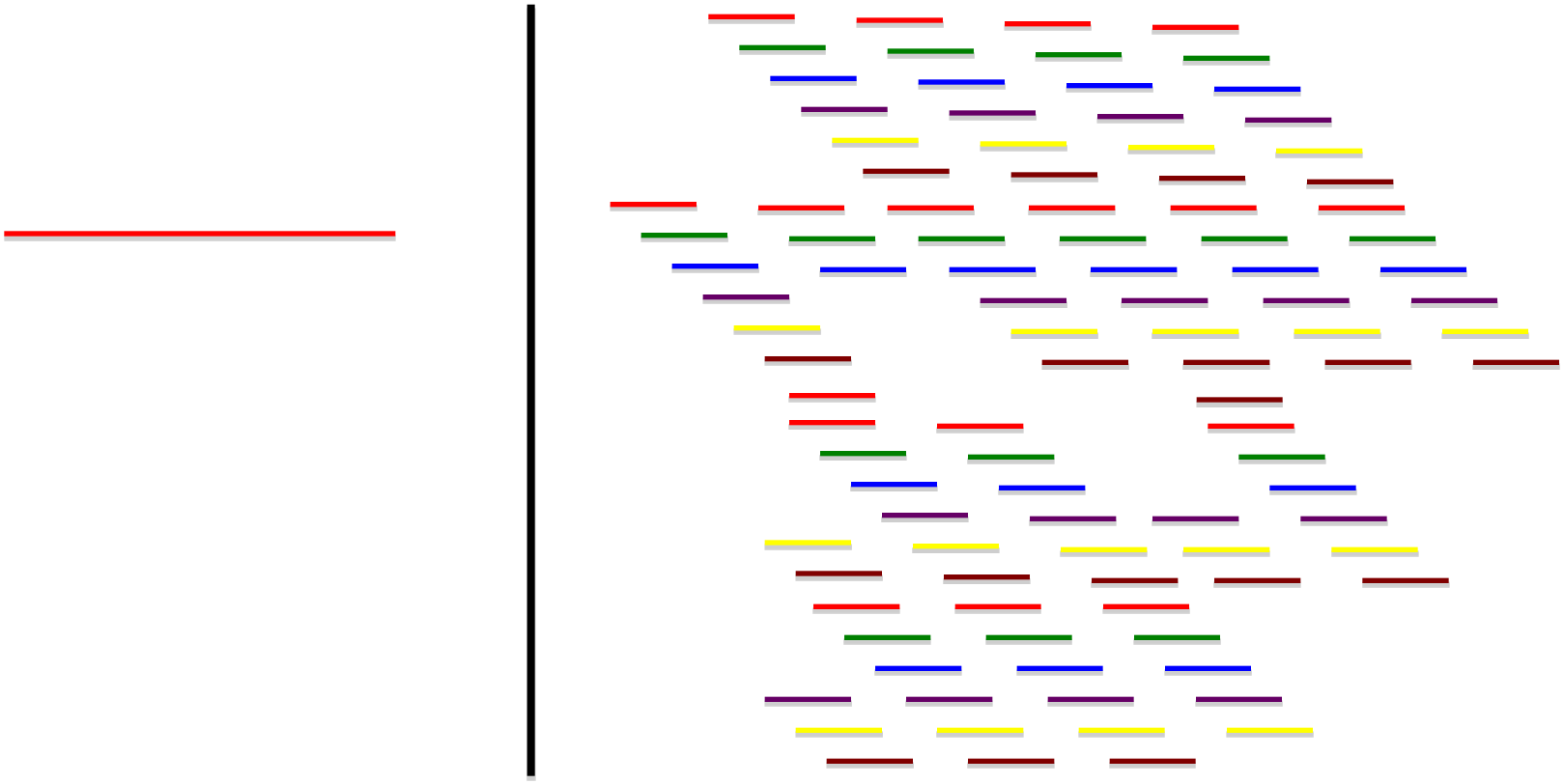
- For biomarker research a refined understanding of the clinical question and a defined translational pathway should be in place prior to commencement



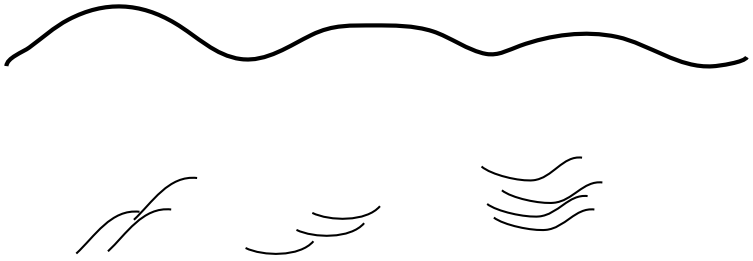
The first disruptive technology in diagnostic pathology since the 1850's

Sanger sequencing

Massively parallel sequencing



Paired-End Sequencing



Primary RNA or DNA

Fragment at random and sequence both ends of each DNA fragment

Align fragments to reference genome sequence

ATGCCGCG
ATGTCGCG

single nucleotide variants (SNVs)

ATCGG..CGGATG
ATCGG..TATTCA

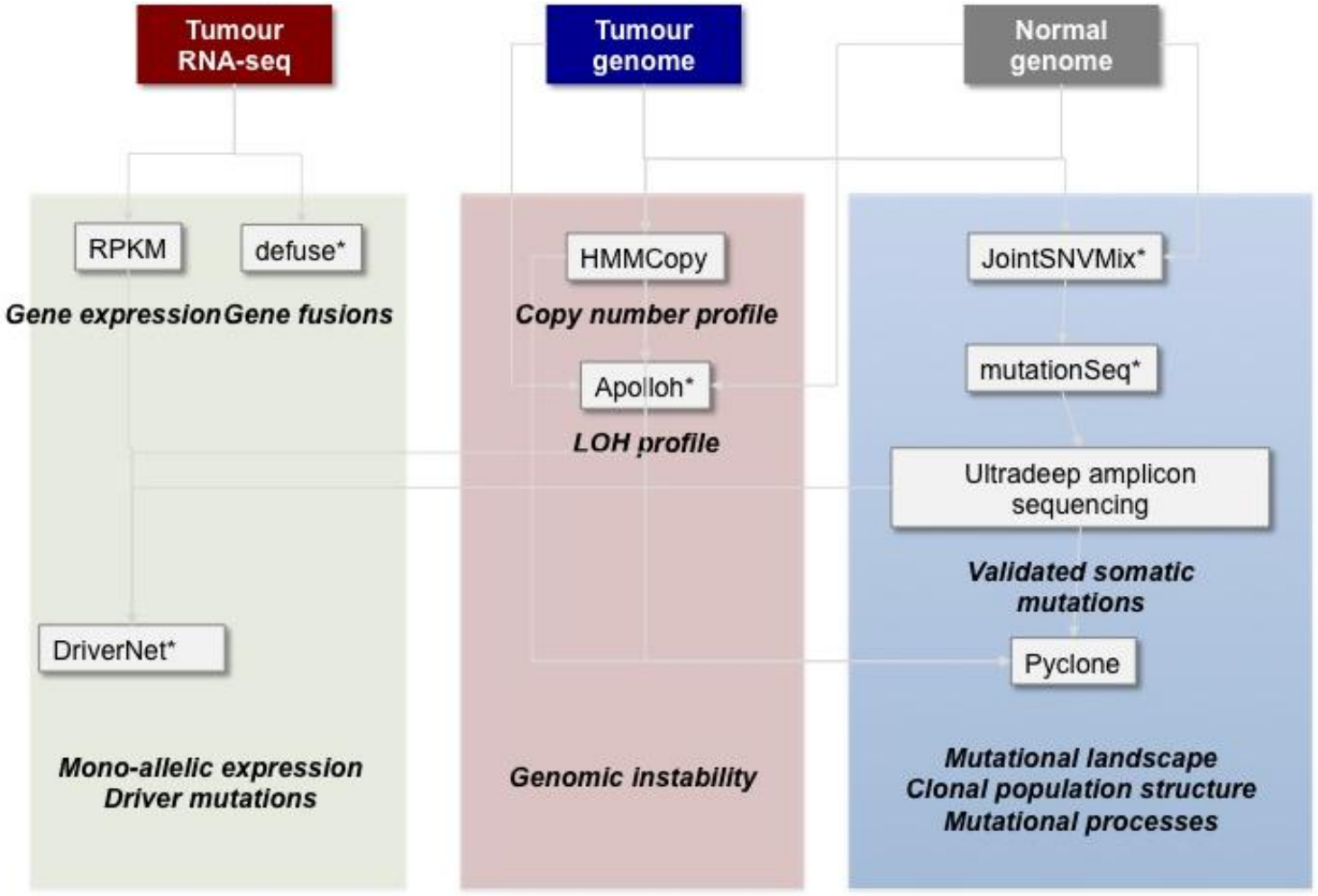
chr20 chr11
Translocations, inversions

ATCGGCGGATG
ATCGG--GATG

indels

SNV's called with SNV mix Goya et al. Bioinf, 2010

Computational interpretation of cancer genomes



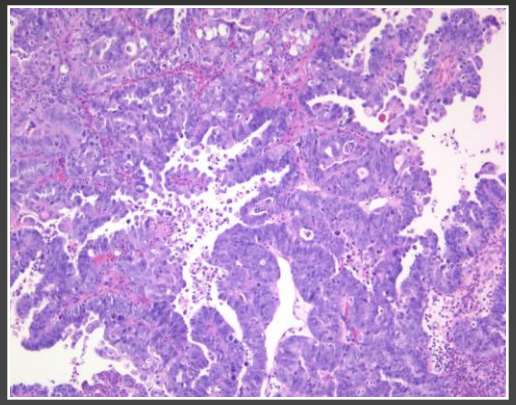
What to sequence?

WHAT DO YOU WANT TO LEARN?

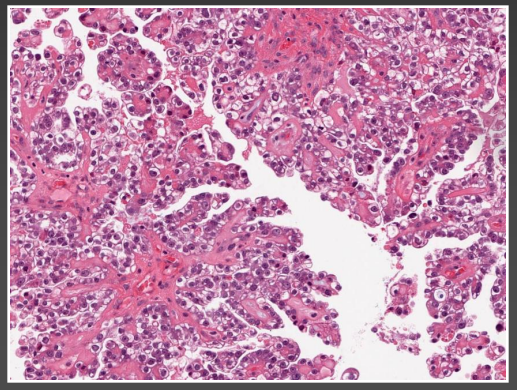
Note
Powerful technology can not overcome poor experimental design

Three flavors of cancer: an approach to cancer genomics

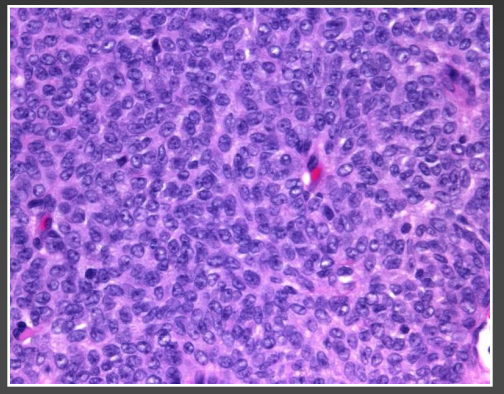
- High grade cancers
- High grade serous cancer
- Pathognomonic mutations unlikely



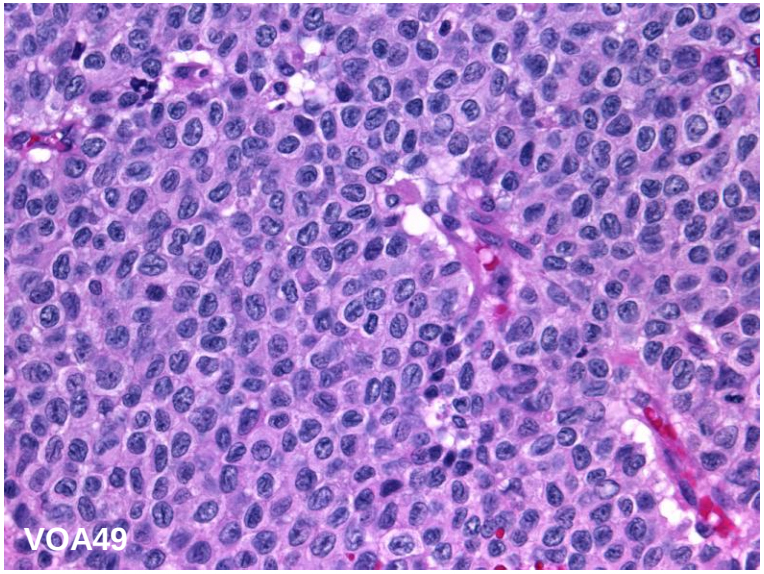
- Moderate grade cancers
- Clear cell cancer
- Mutations in specific pathways that will be important in other cancers

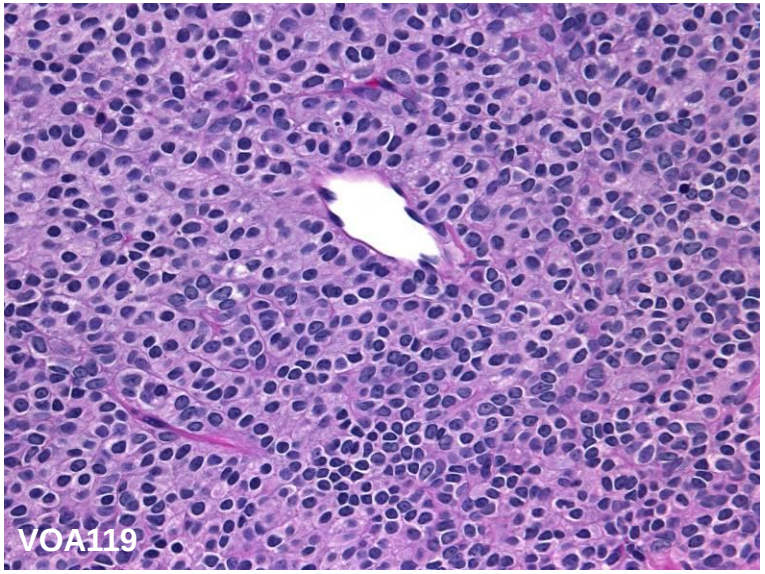


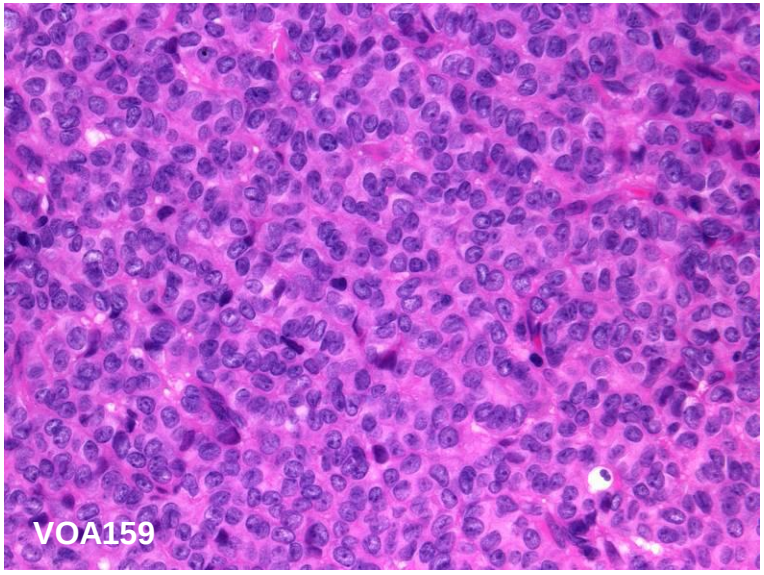
- Unusual tumours with pathognomonic features
- Granulosa cell tumor of the ovary
- Pathognomonic mutations

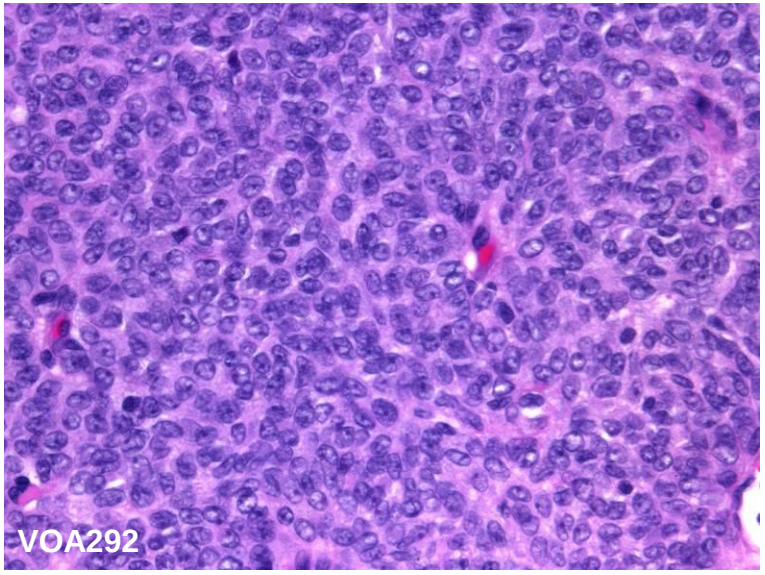


Type 3 Cancers; Example Granulosa cell tumours





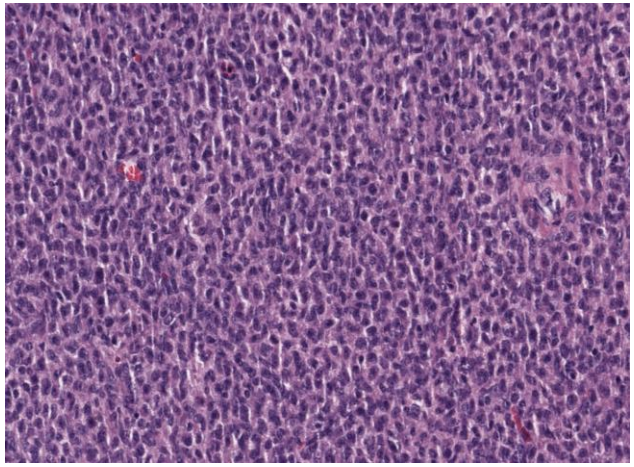


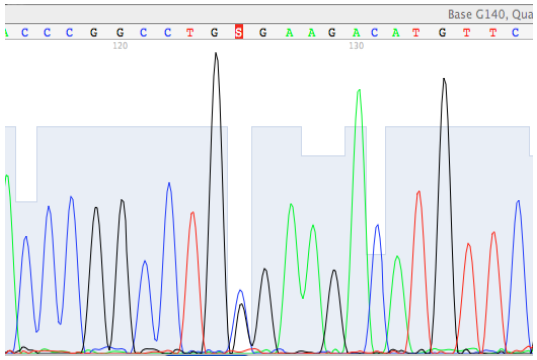


FOXL2 mutation in all 4 granulosa cell tumors of the ovary

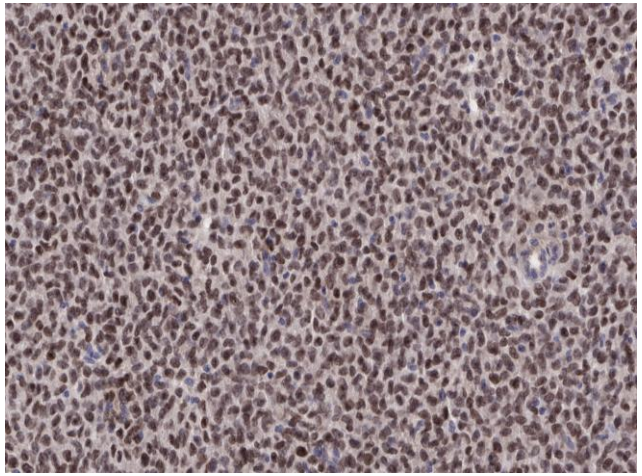
Shah et al (2009) NEJM

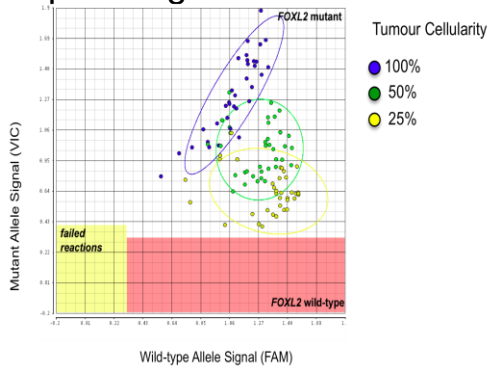
FOXL2 IHC and mutational analysis as a standard diagnostic (Kommoss et al Mod Path in press)





Confirmation of FOXL2 aGCT specific c.402C>G mutation by Sanger Sequencing

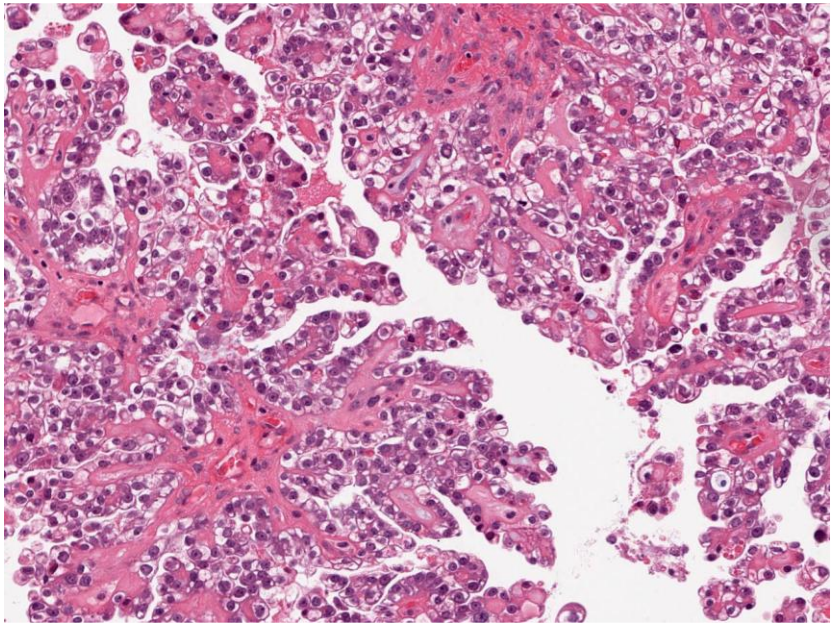




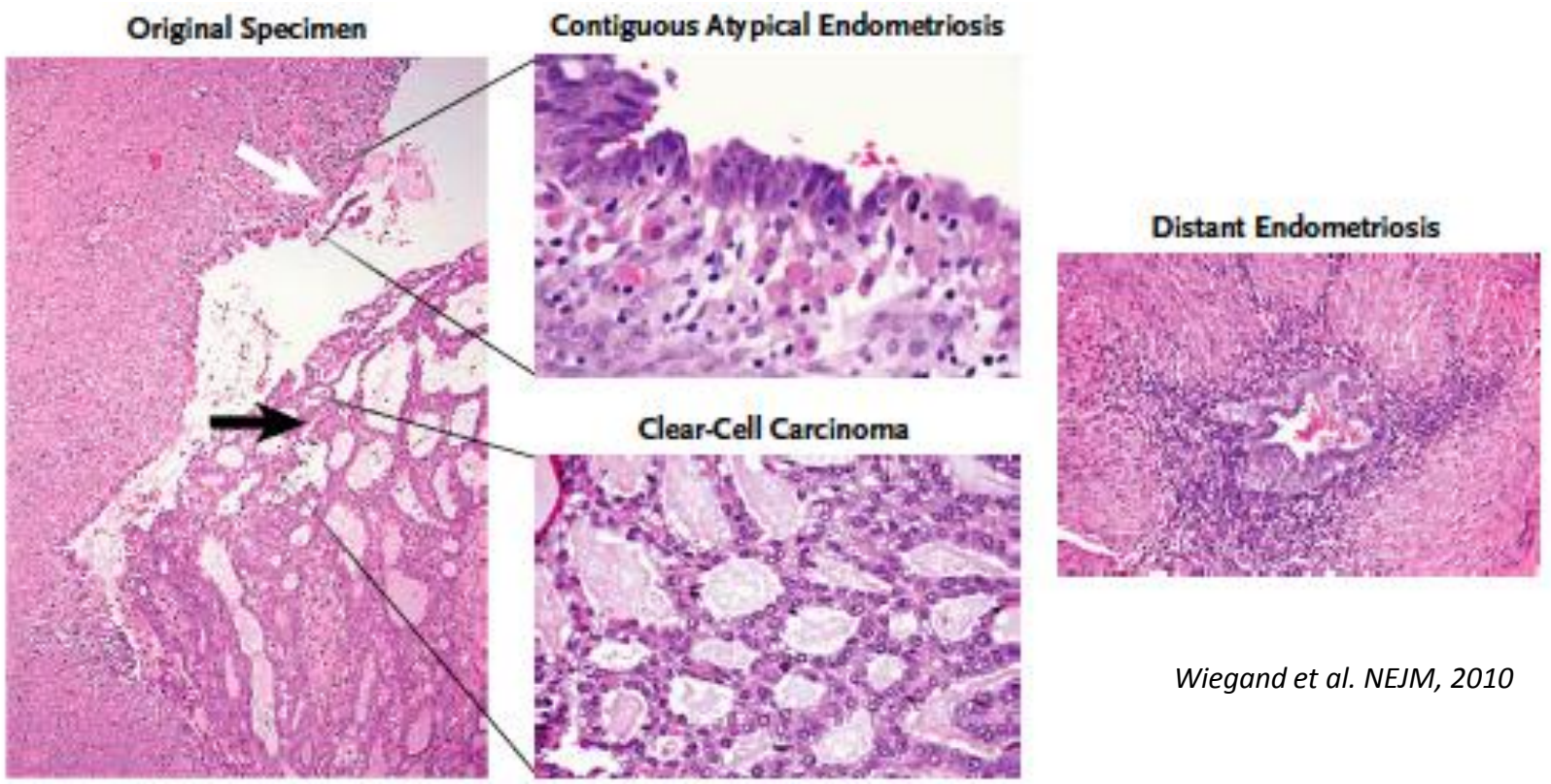
TaqMan based digital mutation assay for FOXL2 aGCT specific c.402C>G mutation

Subtype driven approach to ovarian cancer research: Clear cell carcinoma of the ovary

- 2nd most common ovarian carcinoma subtype in NA (12%) and more frequent in Asia
- Do not respond to standard ovarian chemotherapy
- No other treatments available
- Molecular basis little understood
- Weird cousins of renal CCC
- Relatively genominally stable (hence small study should yield)

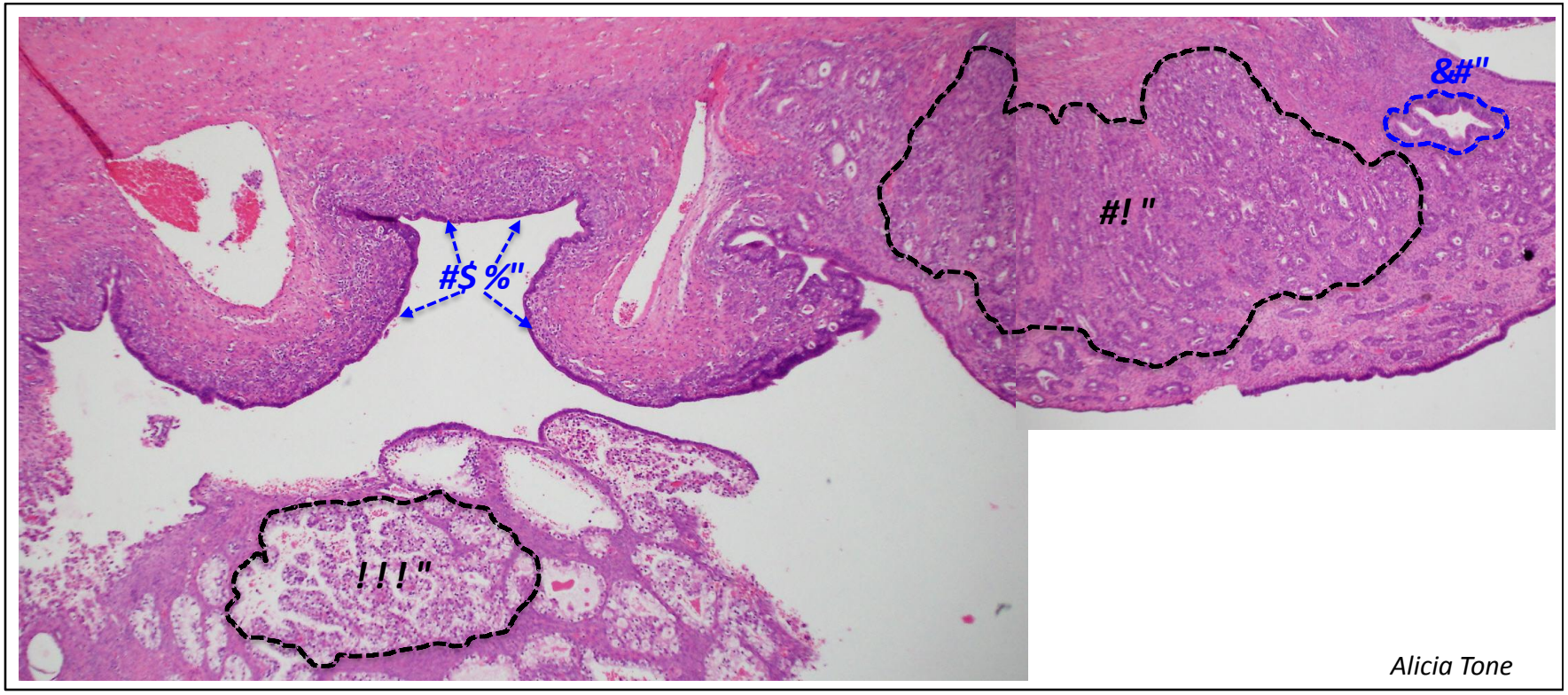


- Clear cell carcinomas are strongly associated with endometriosis
- ARID1A mutations predate the transformation of endometriosis into cancer



Wiegand et al. NEJM, 2010

Question 1: Beyond PIK3CA, ARID1a and MET, what events cause endometriosis to turn bad?



Question 2: prognostic markers for stage 1 OCCC

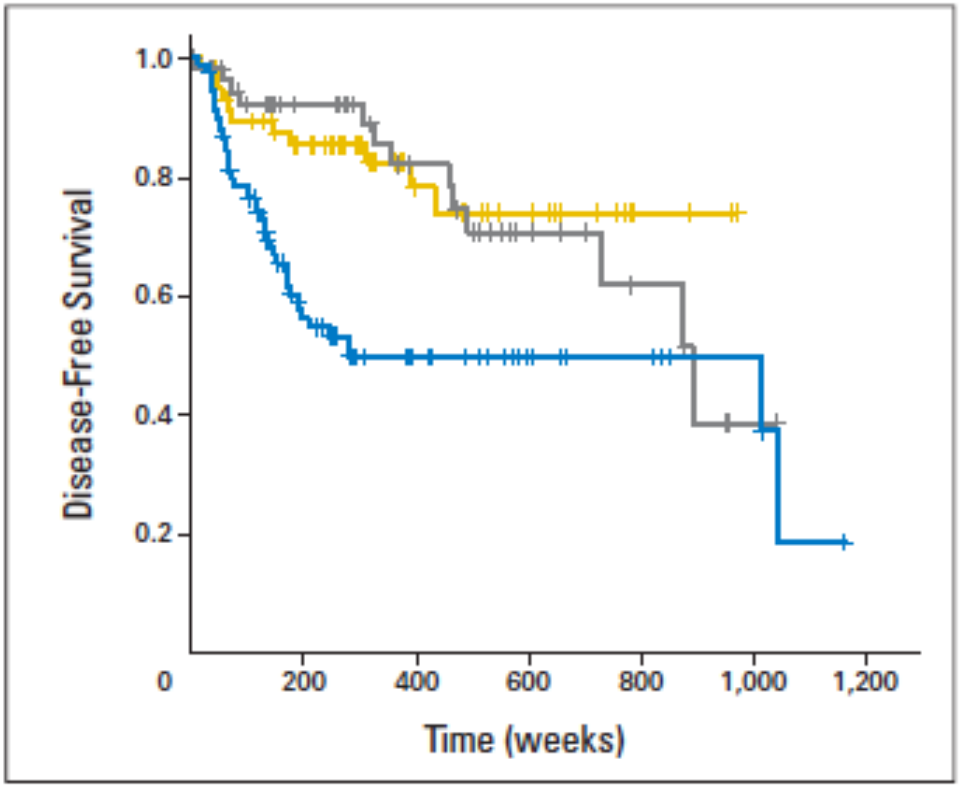


Fig 2. Disease-free survival in stage IA/IB and IC (gold, stage IA, n = 60; gray, stage IC with rupture alone, negative surface involvement, and negative cytology, n = 56; blue, stage IC other [includes unknown/positive cytology and unknown/positive surface involvement], n = 91).

P Hoskins et al JCO 2012

Question 3: markers for radiation response IC and II

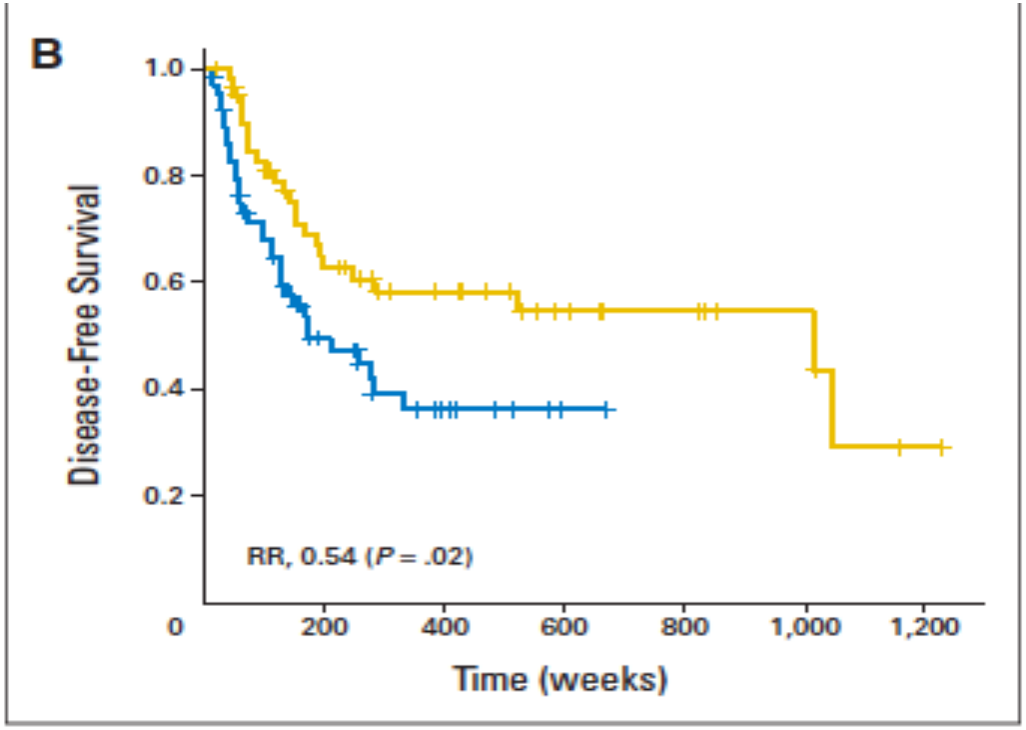
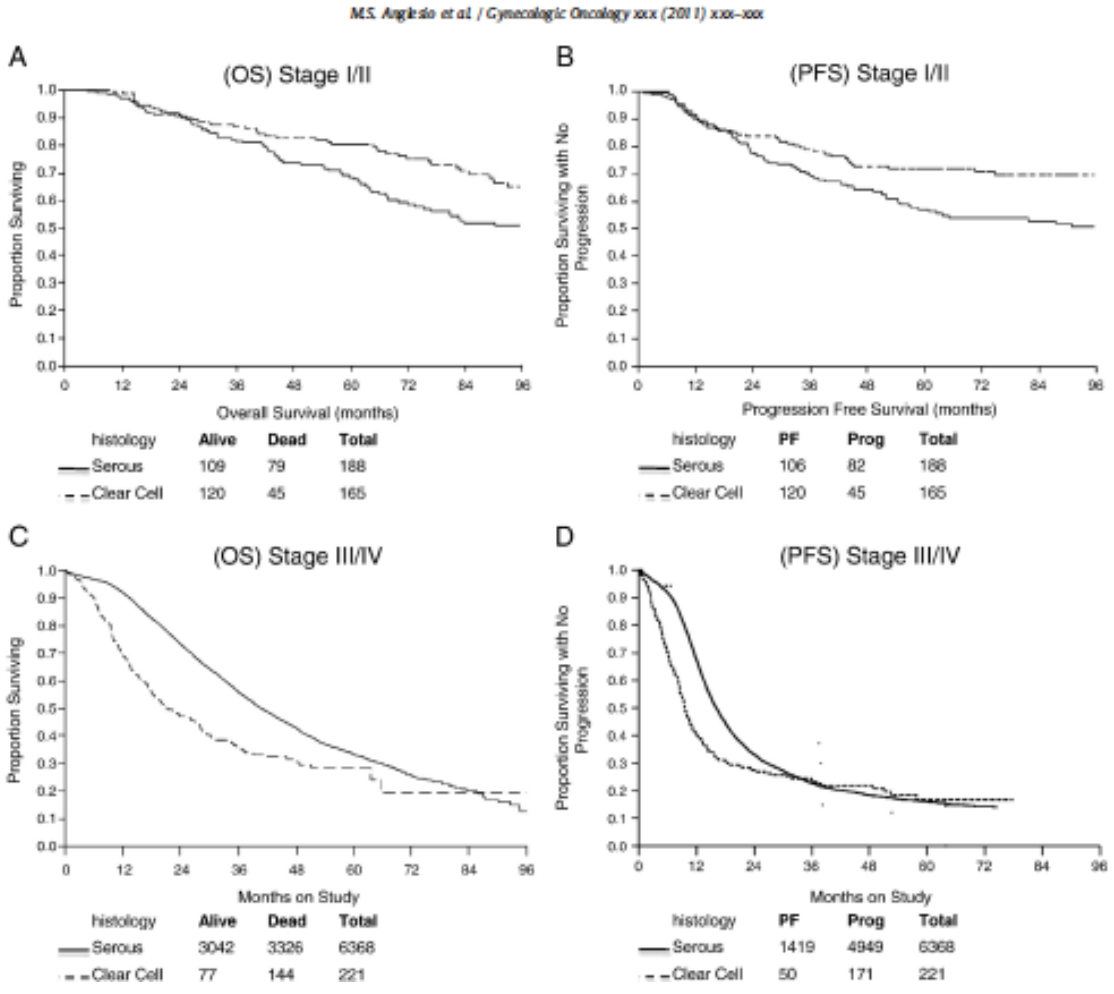


Fig 4. Impact of irradiation. (A) Stage IA/B and IC defined by rupture alone (gold, with irradiation, n = 57; blue, no irradiation, n = 63). (B) All other stage IC and stage II (gold, with irradiation, n = 59; blue, no irradiation, n = 62). RR, relative risk.

P Hoskins et al JCO 2012

Ovarian CCC outcomes



Question 4: how to treat late stage OCCC

- New therapeutic targets needed
- Targets need evaluation in CCC context (CCC model systems. Mike Anglesio)
- Could ARID1A loss uncover an effective target (Kim Wiegand)

K Wiegand, Poster S1, #272
M Anglesio, Poster S1, #14

Subtype specific cell line models: essential for translation of subtype focused research

Cell Line	Reported Histotype in Literature	COSP Markers									COSP Prediction (Clinical)				Mutational Profile	Validated Histotype based on immuno- and mutational profiles
		p16 (CDKN2A)	MDM2	TFF3	p53 (TP53)	VIMENTIN	WT1	HNF1B	PR	DKK1	CCC	ENOCa	HGSC	MUC		
JHOC-5	CCC	1	0	0	1	1	0	1	0	0	***85***	13	2	0	none detected	**CCC**
JHOC-7	CCC	1	1	0	1	1	0	1	0	0	***99***	1	0	0	PIK3CA	**CCC**
JHOC-9	CCC	1	0	0	1	1	0	1	0	0	***85***	13	2	0	PIK3CA/ARID1A	**CCC**
RMG-2	CCC	0	1	0	1	1	0	1	0	1	***97***	3	0	0	PPP2R1A/ARID1A	**CCC**
TOV21G	CCC	0	0	0	1	1	0	1	0	1	***55***	41	4	0	KRAS/PTEN/PIK3CA/ARID1A	**CCC**
VOA782_XL	ENOCa	0	1	0	1	1	0	1	0	0	***100***	0	0	0	PIK3CA/ARID1A	**CCC**
A2780	Adenocarcinoma	0	0	0	1	1	0	0	0	1	0	***94***	6	0	PTEN/ARID1A	**ENOCa**
ES-2	CCC	1	0	0	1	1	0	0	0	1	0	***100***	0	0	BRAF	**ENOCa**
IGROV1	Mixed	1	0	0	1	1	0	1	0	1	17	***82***	1	0	ARID1A	**ENOCa**
TOV112D	ENOCa	0	0	0	2	1	0	0	0	1	0	38	***62***	0	CTNNB1	**ENOCa****
SKOV3	carcinoma	0	0	X	0	0	0	1	1	X	***50% ENOCa (incomp.)***				PIK3CA/**ARID1A**	**ENOCa**
ov2008	ENOCa	1	0	0	0	0	1	0	0	1	0	0	***100***	0	PIK3CA	**HGSC**
CAOV3	Adenocarcinoma	1	0	0	0	0	0	0	0	1	0	0	***100***	0	none detected	**HGSC**
Kuramochi	Undifferentiated	1	1	0	2	1	1	0	0	0	0	3	***97***	0	none detected	**HGSC**
OVCAR-4	Serous Adenocarc.	0	0	0	0	1	0	0	0	1	0	0	***100***	0	none detected	**HGSC**
OVCAR-5	Adenocarcinoma	0	0	0	0	0	0	0	0	0	0	0	***100***	0	KRAS	**HGSC**
OVCAR-8	Adenocarcinoma	0	0	0	2	1	0	0	0	1	0	38	***62***	0	none detected	**HGSC**
RMG-1	CCC	0	0	0	0	0	0	1	0	1	22	0	***76***	3	none detected	**HGSC**
VOA1056_CL	LGSC	0	0	0	1	1	0	0	0	0	0	39	***61***	0	NRAS	**LGSC***

OVCARE
BC'S OVARIAN CANCER RESEARCH TEAM

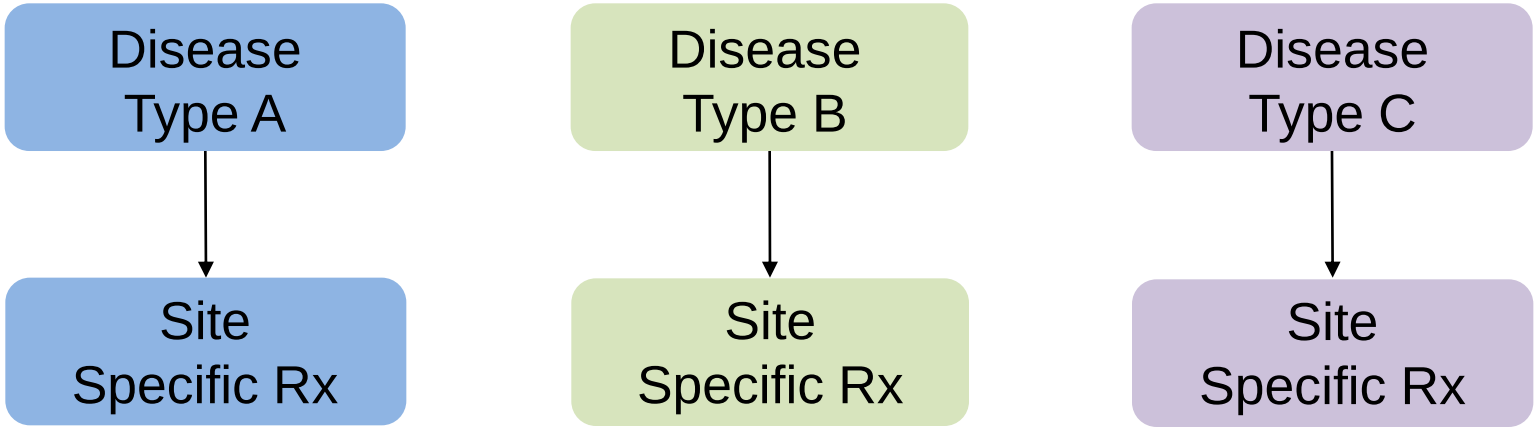
M Anglesio, Tuesday, 8-10, Ballroom C
Poster S1, #14

Changing Paradigms for Cancer Treatment

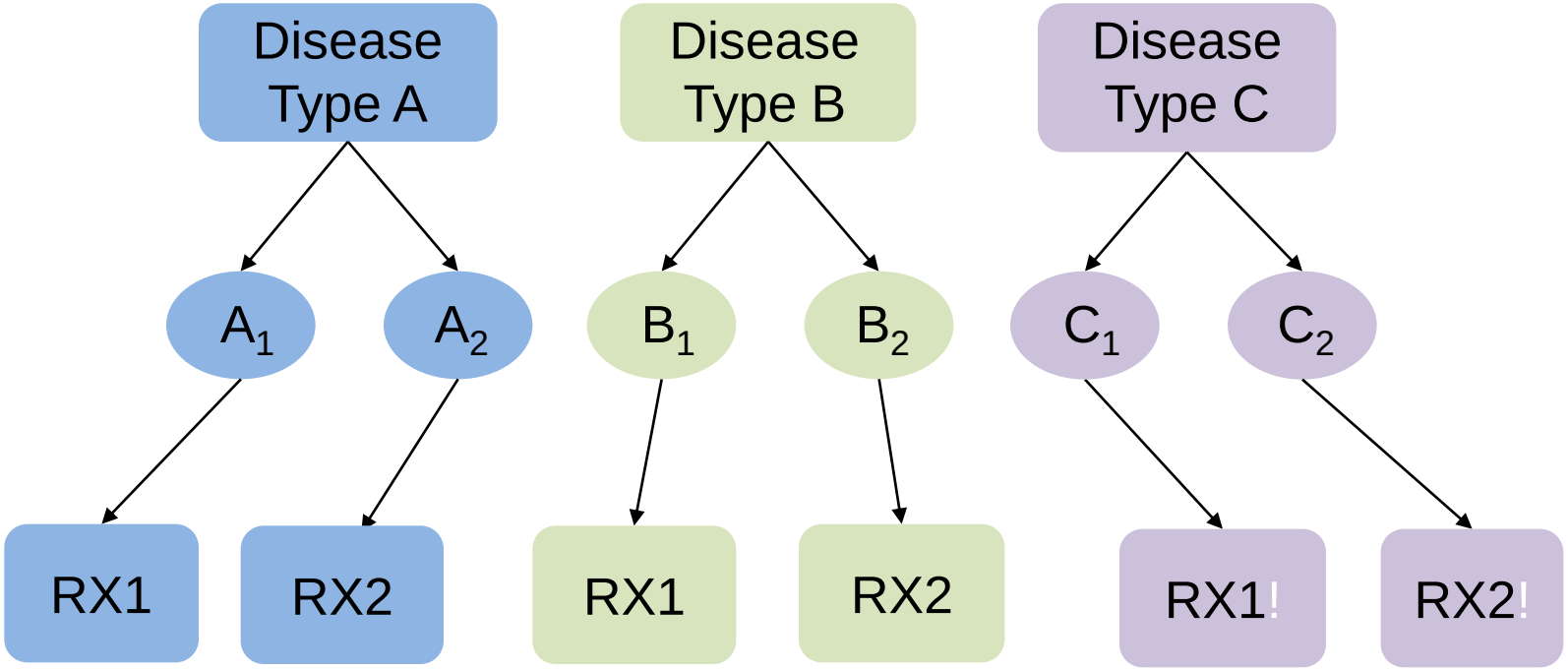
Generic Cancer Treatment	Stratified Cancer Treatment	Individualized Cancer Treatment
1990	2010	? 2020

Breast cancer
Lymphoma
Uterine cancer
Ovarian cancer
Pancreatic cancer

Generic Site Based Cancer Treatment



Stratified Cancer Treatment: 2000-20?? AD



Many successes but too crude

Gastric Cancer: 200 cases analyzed for 700 common mutations: Kennecke, Lim , Yip and Huntsman (funded by BCCF)

Ion AmpliSeq™ Cancer Panel: Content

46 genes, 739 mutations

KRAS	BRAF	EGFR	TP53	PIK3CA	CSF1R	JAK2
NRAS	PTPN11	ERBB2	SRC	FGFR3	NPM1	CDKN2A
RET	HNF1A	SMAD4	GNAS	PDGFRA	MPL	ABL1
PTEN	FLT3	STK11	SMARCB1	KIT	MET	NOTCH1
FGFR2	RB1	JAK3	VHL	KDR	SMO	
HRAS	AKT1	ALK	MLH1	FBXW7	ERBB4	
ATM	CDH1	IDH1	CTNNB1	APC	FGFR1	

National Cancer Institute

Oregon Health & Science University

BCM Baylor College of Medicine

CGL Cancer Genetics Laboratory

Yale

Cancer Research UK

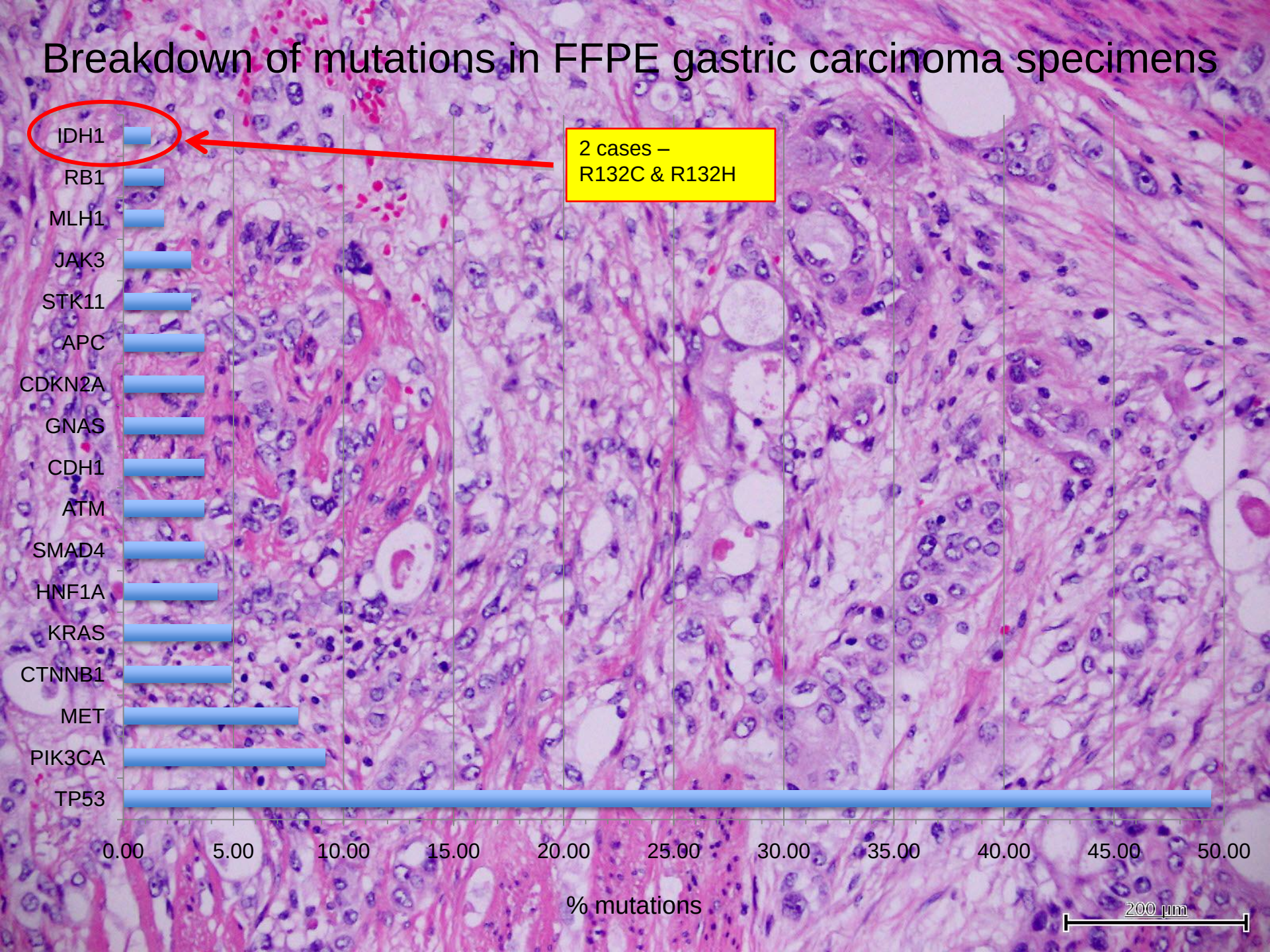
Addenbrooke's Hospital NHS

Cambridge University Hospitals NHS

Foundation Medicine

ion torrent by Life technologies

Breakdown of mutations in FFPE gastric carcinoma specimens



Application of full scale genomics in the clinic

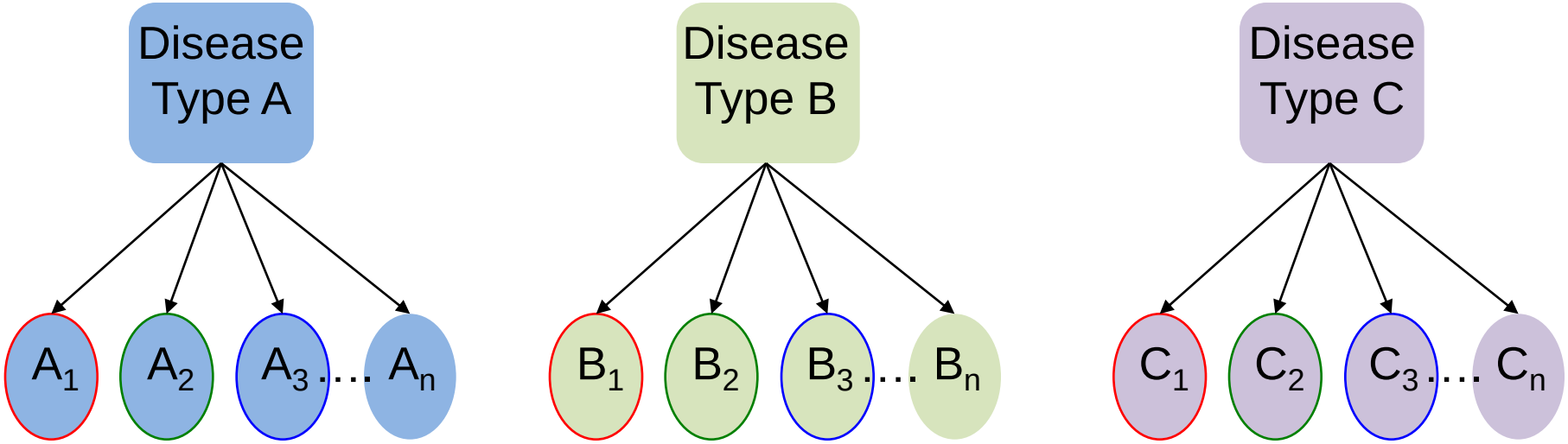
Jones *et al. Genome Biology* 2010, **11**:R82
http://genomebiology.com/2010/11/8/R82

RESEARCH Open Access

Evolution of an adenocarcinoma in response to selection by targeted kinase inhibitors

Steven JM Jones[1*], Janessa Laskin[2], Yvonne Y Li[1], Obi L Griffith[1], Jianghong An[1], Mikhail Bilenky[1], Yaron S Butterfield[1], Timothee Cezard[1], Eric Chuah[1], Richard Corbett[1], Anthony P Fejes[1], Malachi Griffith[1], John Yee[3], Montgomery Martin[2], Michael Mayo[1], Nataliya Melnyk[4], Ryan D Morin[1], Trevor J Pugh[1], Tesa Severson[1], Sohrab P Shah[4,5], Margaret Sutcliffe[2], Angela Tam[1], Jefferson Terry[4], Nina Thiessen[1], Thomas Thomson[2], Richard Varhol[1], Thomas Zeng[1], Yongjun Zhao[1], Richard A Moore[1], David G Huntsman[3], Inanc Birol[1], Martin Hirst[1], Robert A Holt[1], Marco A Marra[1]

Stratified Cancer Treatment 2012 and the promise of genomics—finer stratification

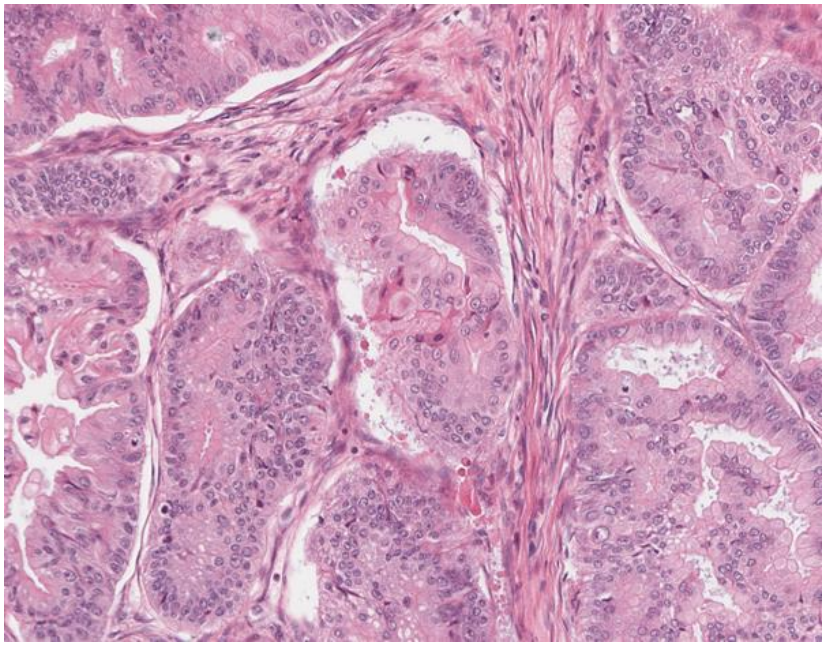


Stratification defined by molecular features

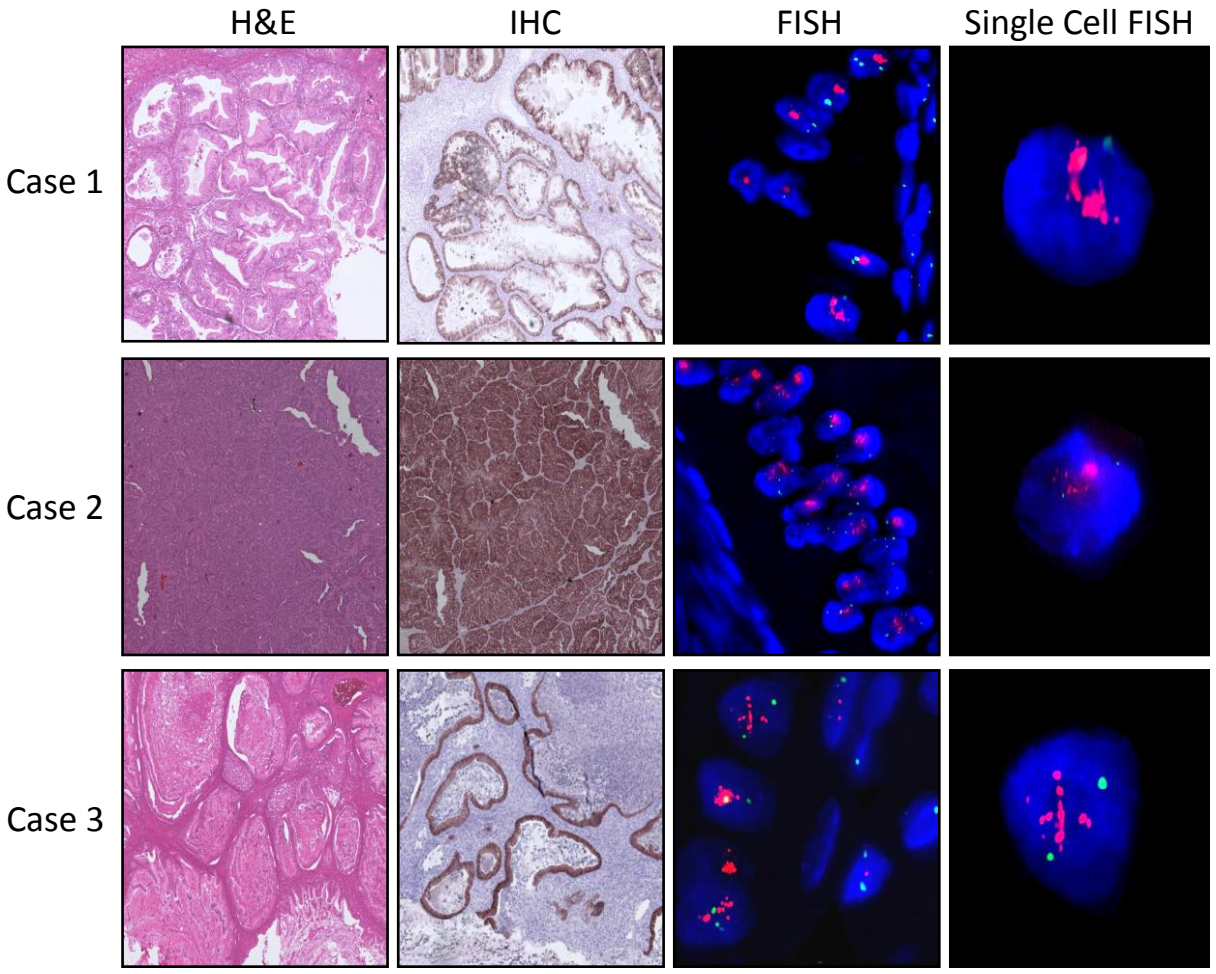
Problems: How many subtypes are there and how to test treatment options?

Mucinous Carcinomas of the Ovary

How to treat relapsed mucinous carcinomas (do not respond to current therapy?)



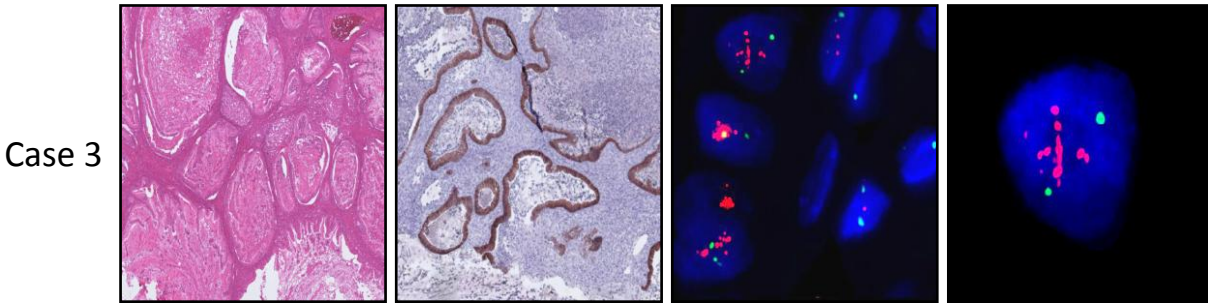
HER-2 amplification seen in 22% of Mucinous ovarian carcinomas



McAlpine BMC Cancer, 2010 and Anglesio J Path 2012

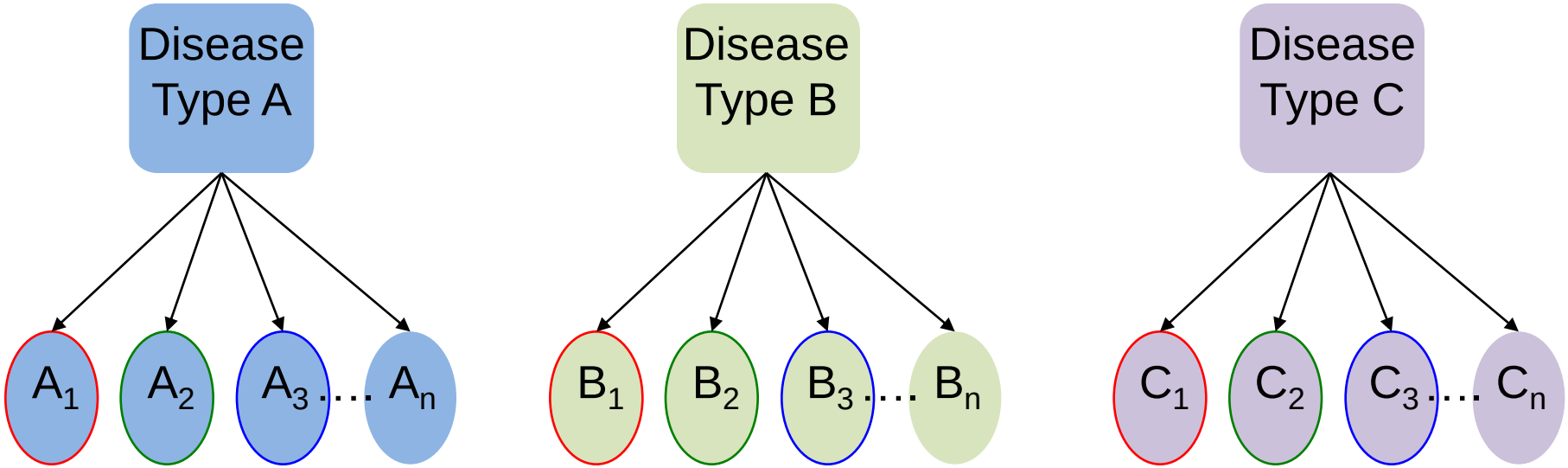
Example: HER-2 is amplified in 20% of mucinous carcinomas, anecdotal evidence that these cancers respond to Trastuzumab

Problem: How do you mount a trial for 20% of 4% of ovarian cancers?



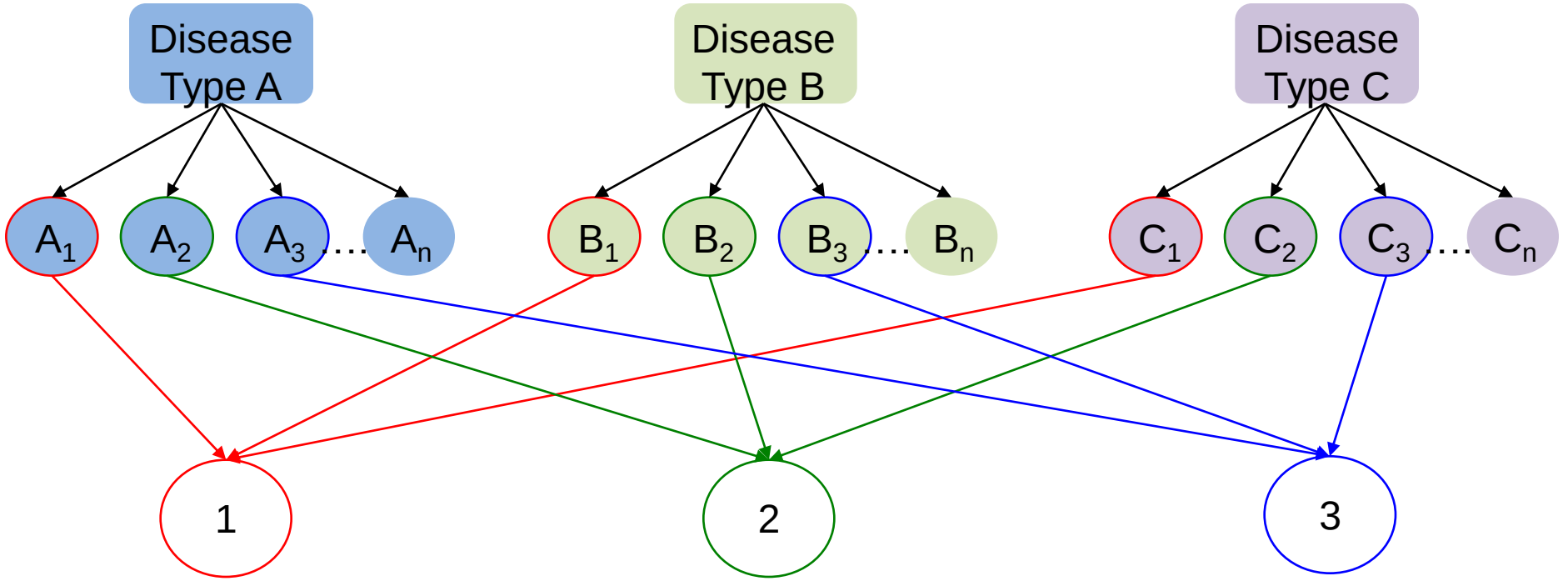
McAlpine BMC Cancer, 2010

Stratified Cancer Treatment 2012 and the promise of genomics – finer stratification



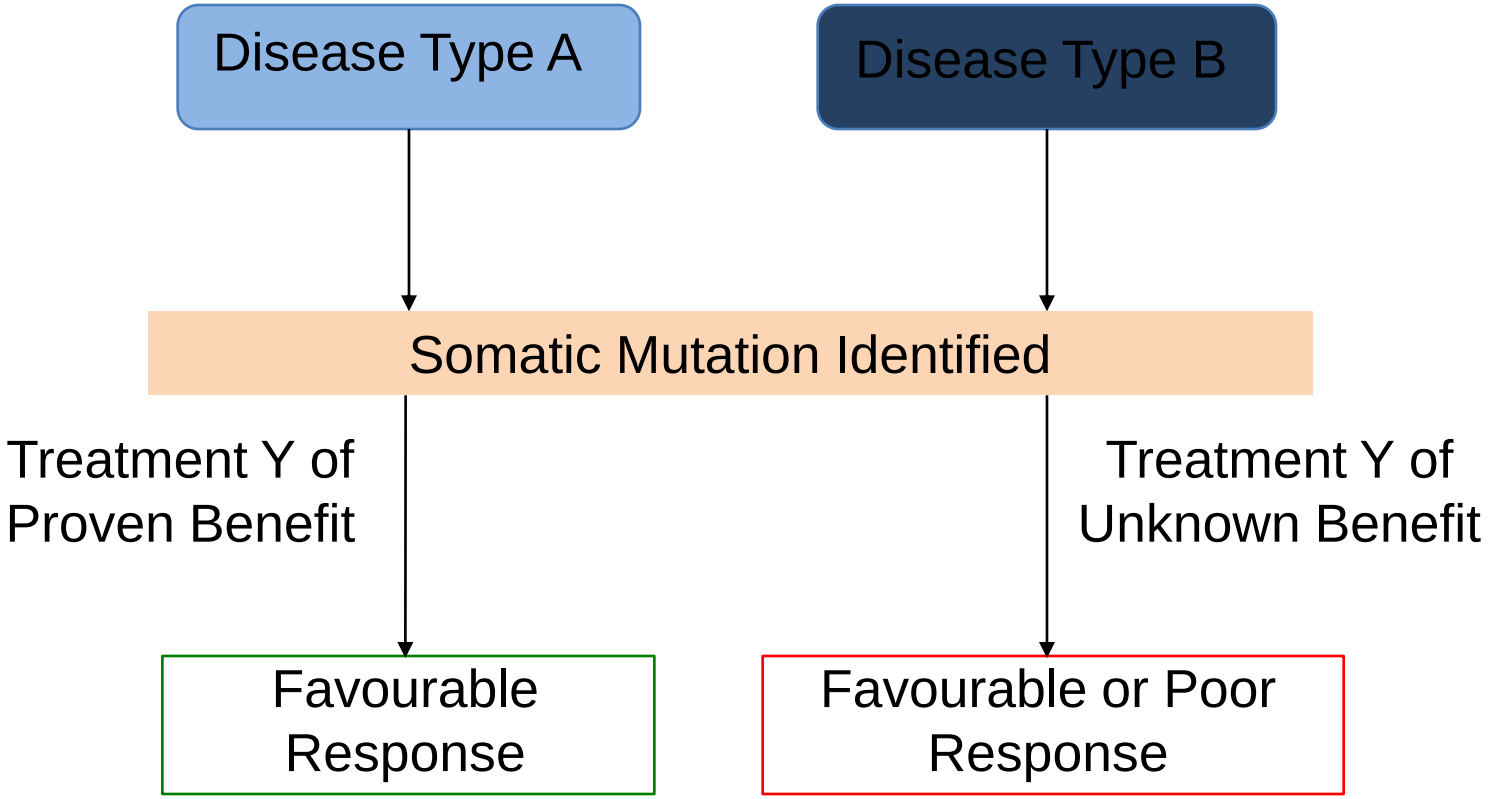
Fortunately targetable molecular features used to stratify are often shared between different cancers types.

Is a mutation based taxonomy for cancers possible or desirable?



Advantage: tractable sized patient populations to test treatments

Mutation-Based Treatment Stratification



Challenges (beyond finding the right drug to match a mutation/feature) of using heterogeneous molecular features to drive treatment

1. Intratumoral heterogeneity- is the mutation clonally dominant in the cancer today?
2. Is the mutation active in the cancer?
3. Is the mutation targetable in the context of the cellular origins of the cancer?

Just when we thought it was safe to go back to the water

Cancer: A tale of many Genomes

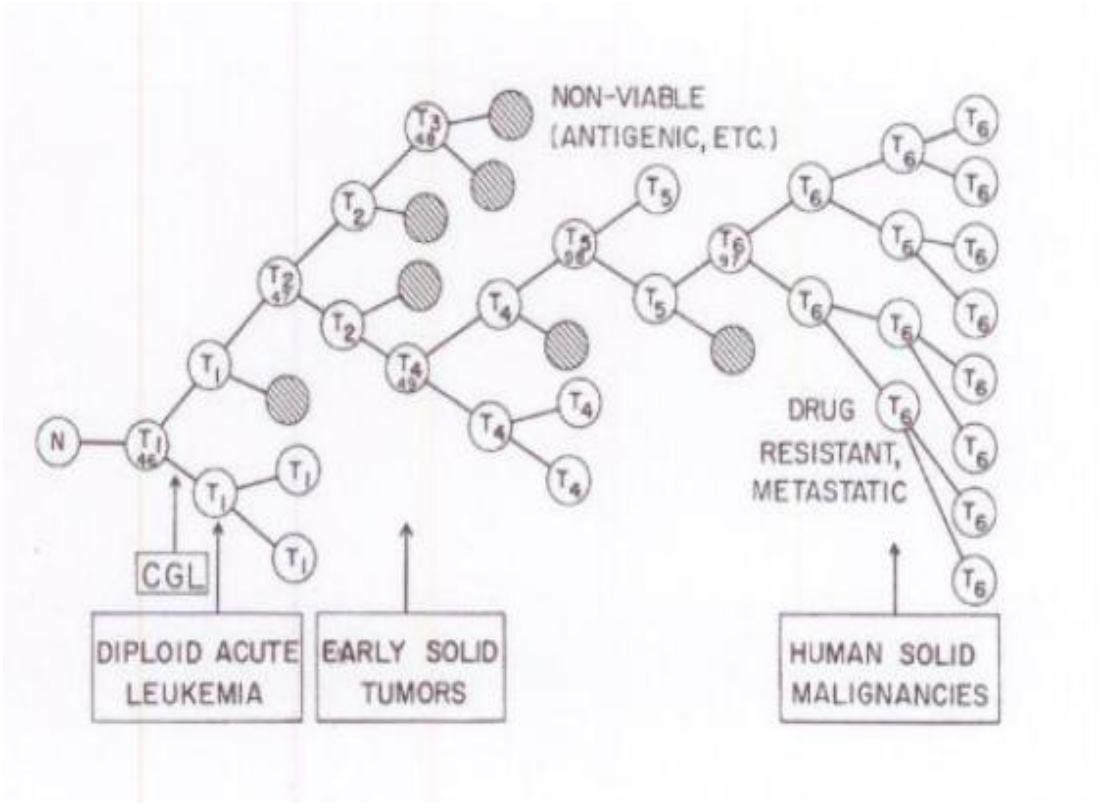
Germline SNP's and mutations

Somatic mutations

Germline Genome

Cancer Genomes

The clonal evolution of tumor cell populations: Peter Nowell—Science 1976



Intra-tumoral heterogeneity is better than a new idea it is a road tested idea that has found its time

Intratumoral heterogeneity has implications for how we sample and how we treat cancers

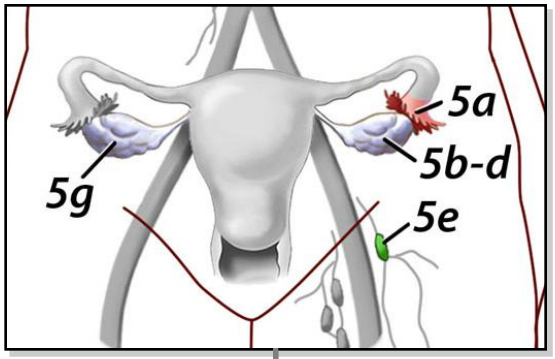
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 8, 2012 VOL. 366 NO. 10

Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Marco Gerlinger, M.D., Andrew J. Rowan, B.Sc., Stuart Horswell, M.Math., James Larkin, M.D., Ph.D., David Endesfelder, Dip.Math., Eva Gronroos, Ph.D., Pierre Martinez, Ph.D., Nicholas Matthews, B.Sc., Aengus Stewart, M.Sc., Patrick Tarpey, Ph.D., Ignacio Varela, Ph.D., Benjamin Phillimore, B.Sc., Sharmin Begum, M.Sc., Neil Q. McDonald, Ph.D., Adam Butler, B.Sc., David Jones, M.Sc., Keiran Raine, M.Sc., Calli Latimer, B.Sc., Claudio R. Santos, Ph.D., Mahrokh Nohadani, H.N.C., Aron C. Eklund, Ph.D., Bradley Spencer-Dene, Ph.D., Graham Clark, B.Sc., Lisa Pickering, M.D., Ph.D., Gordon Stamp, M.D., Martin Gore, M.D., Ph.D., Zoltan Szallasi, M.D., Julian Downward, Ph.D., P. Andrew Futreal, Ph.D., and Charles Swanton, M.D., Ph.D.

Mutational profiling of multi-region anatomic sites



DNA extraction

Affymetrix SNP 6.0

Whole exome sequencing

Deep targeted resequencing

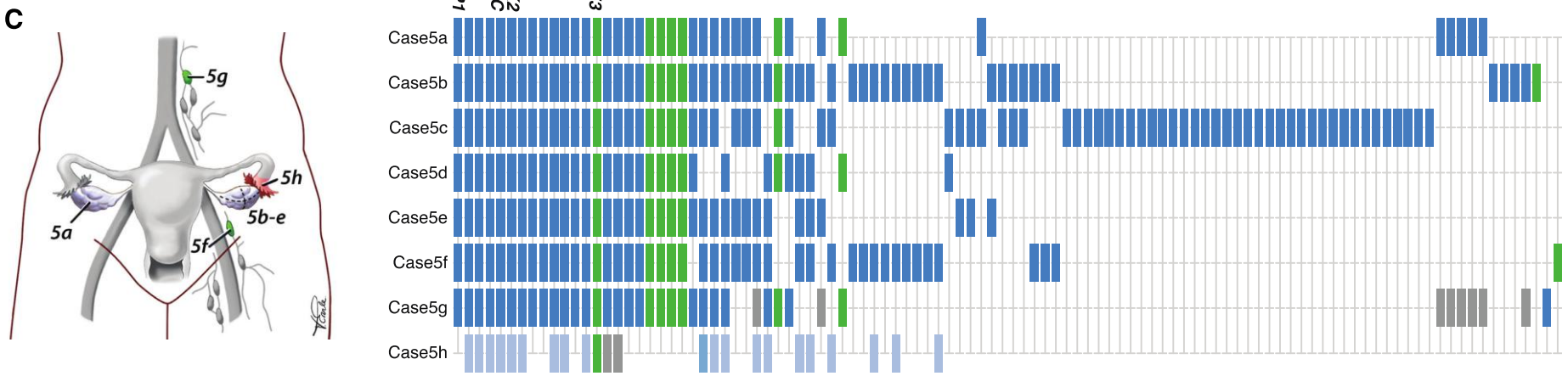
Project lead and Informatics (Dr. S. Shah
Multiregion sampling (Dr. J. McAlpine)
Histopathology (Dr. C.B. Gilks)
Molecular pathology (Dr. D. Huntsman)

Copy number genome architecture

Somatic point mutations

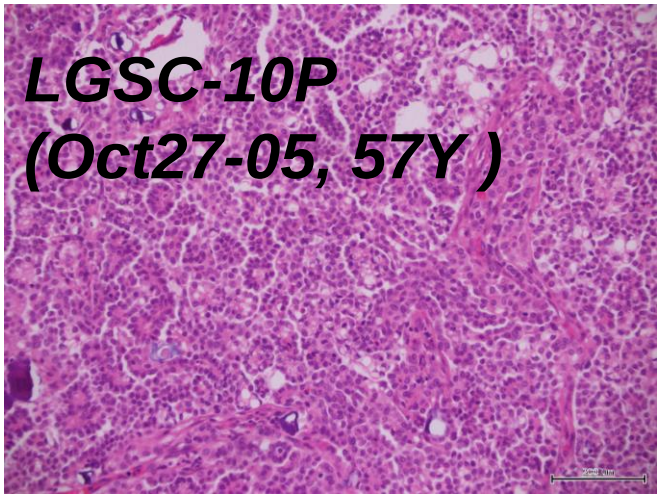
Clonal population structure

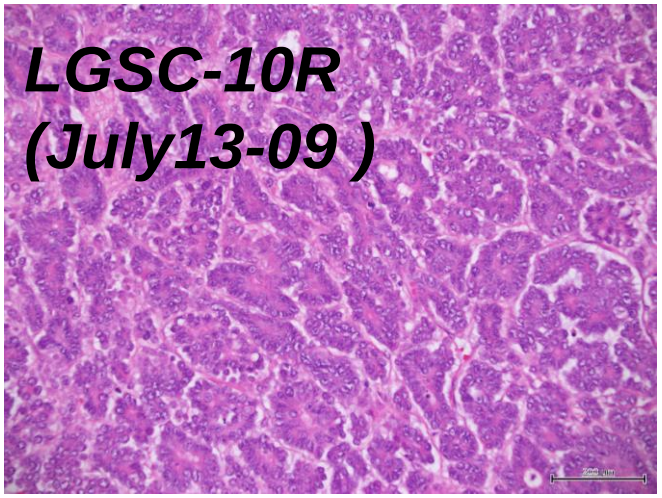
Regional diversity of mutational profiles



- 6 cases complete
- 52% +/- 31% of mutations present in all samples
- 91% in primary-recurrence comparison
- 10% in most diverse case
- *TP53* always in all samples
- Driver mutations *PIK3CA*, *CTNNB1*, *NF1*, *PDGFRB* not present in all samples

Sadly even low grade cancers are heterogeneous





31% (1456 variant/4723 total)

***KRAS**
chr12: 25,398,284 C>A;
p.G12V (COSM520)

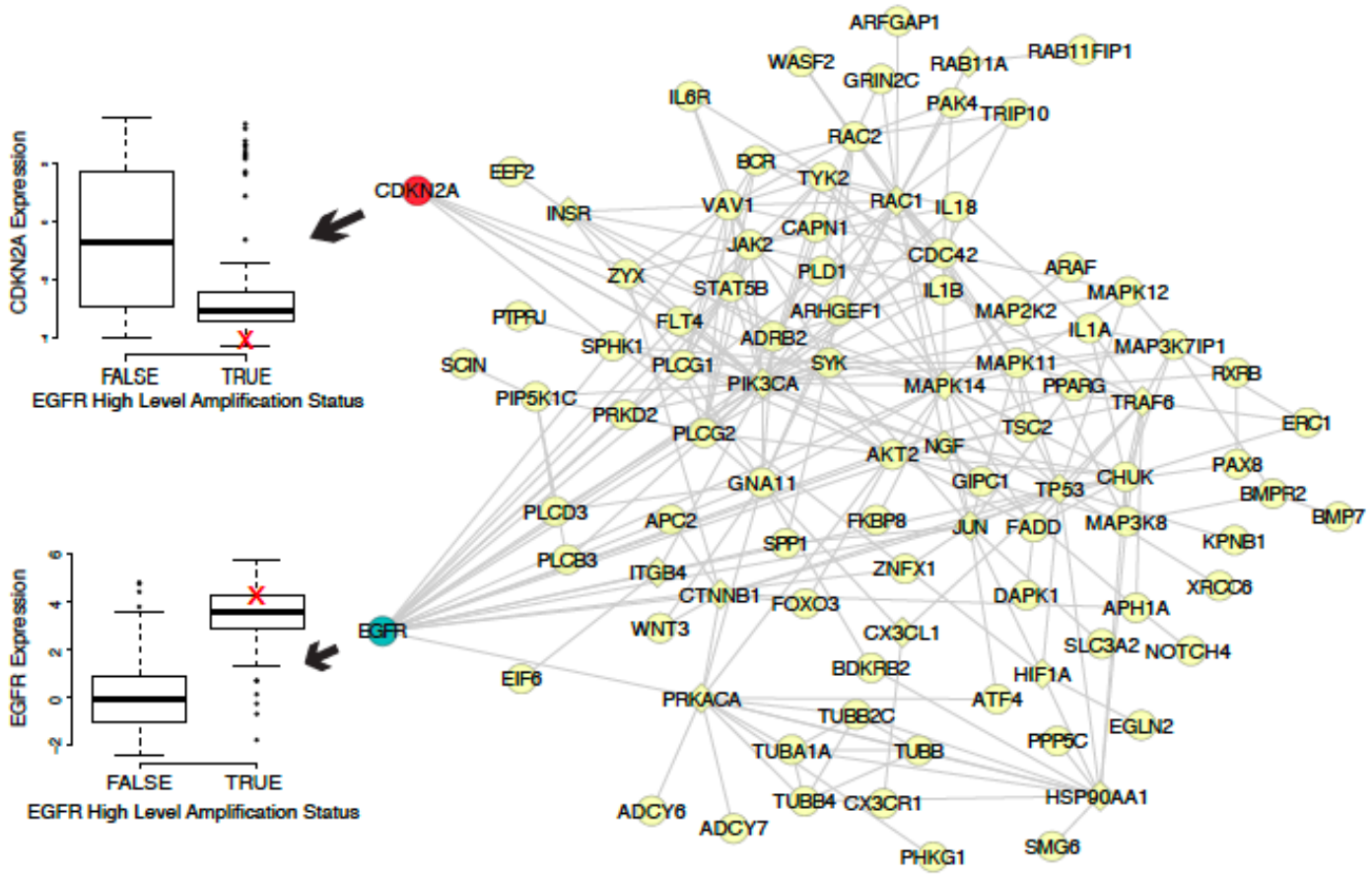
N/A

Is the mutation actively driving the cancer or is it a deadbeat ?

- Mutations may critical in the early development of a cancer may no longer be active
- Mutations that are invariably active in one cancer type may no longer be active in another
- Fortunately tools exist to determine the activation status of mutations in cancer

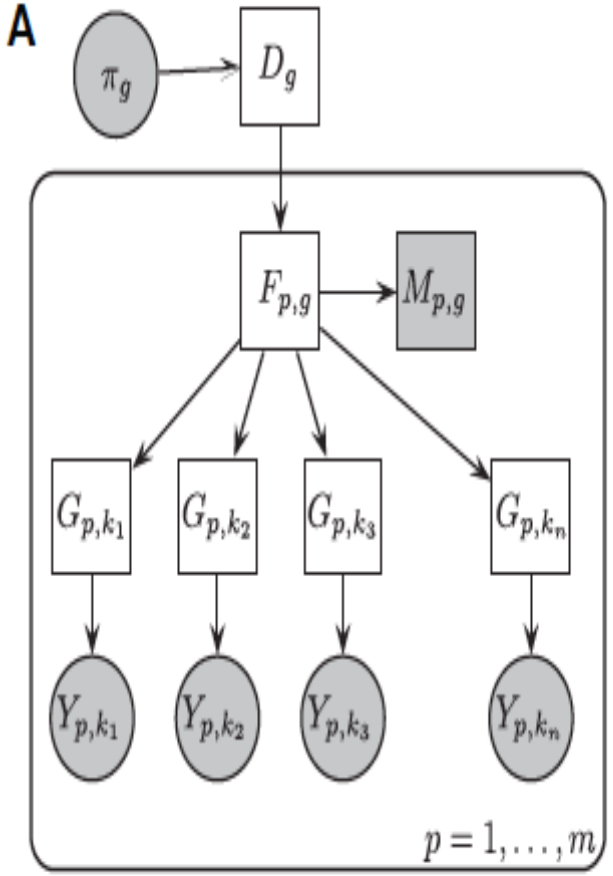
To stop the vehicle –shoot the driver

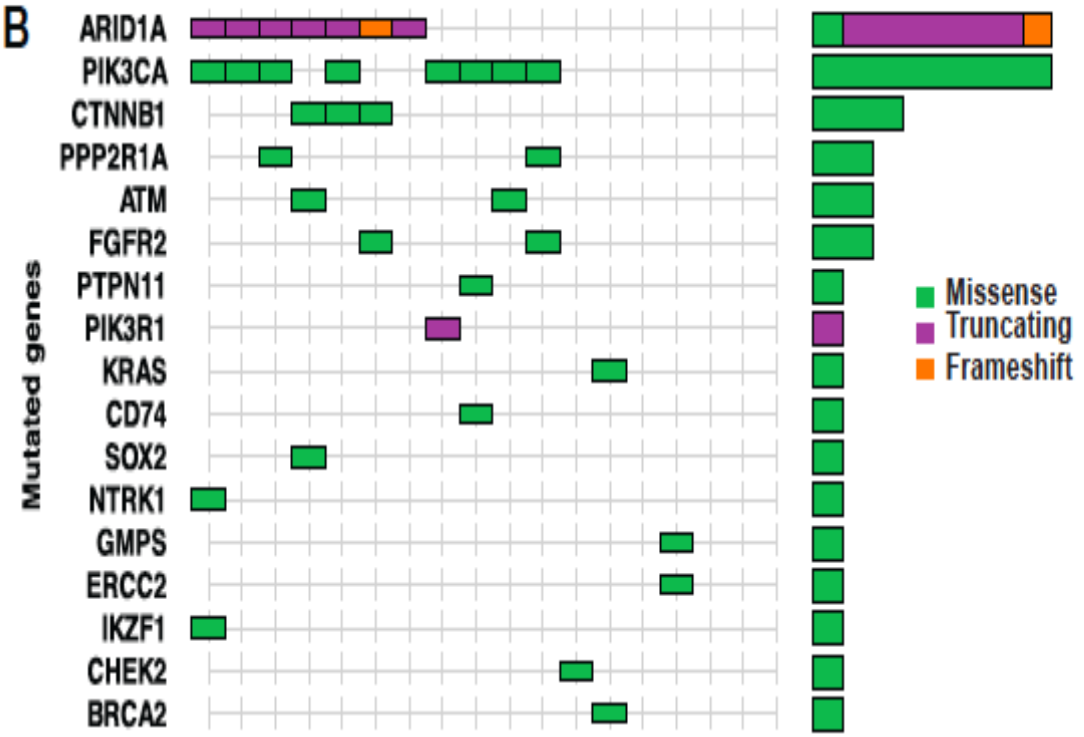
The functional impact of mutations can be detected in expression profiles



Overrepresented pathways of genes exhibiting outlying expression associated with EGFR high level amplification
PI3K signaling: FOXO3,CDKN2A,CHUK,SYK,AKT2,EEF2,ERC1,PPP5C,TSC2
MAPK signaling: CDC42,MAP3K8,IL1B,CHUK,IL1A,AKT2,EGFR,PPP5C,RAC2,MAP2K2,MAPK11,ATF4,MAPK12
EGF receptor signaling pathway: STAT5B,AKT2,EGFR,PRKD2,RAC2,MAP2K2,MAPK11,PLCG1,MAPK12,PLCG2

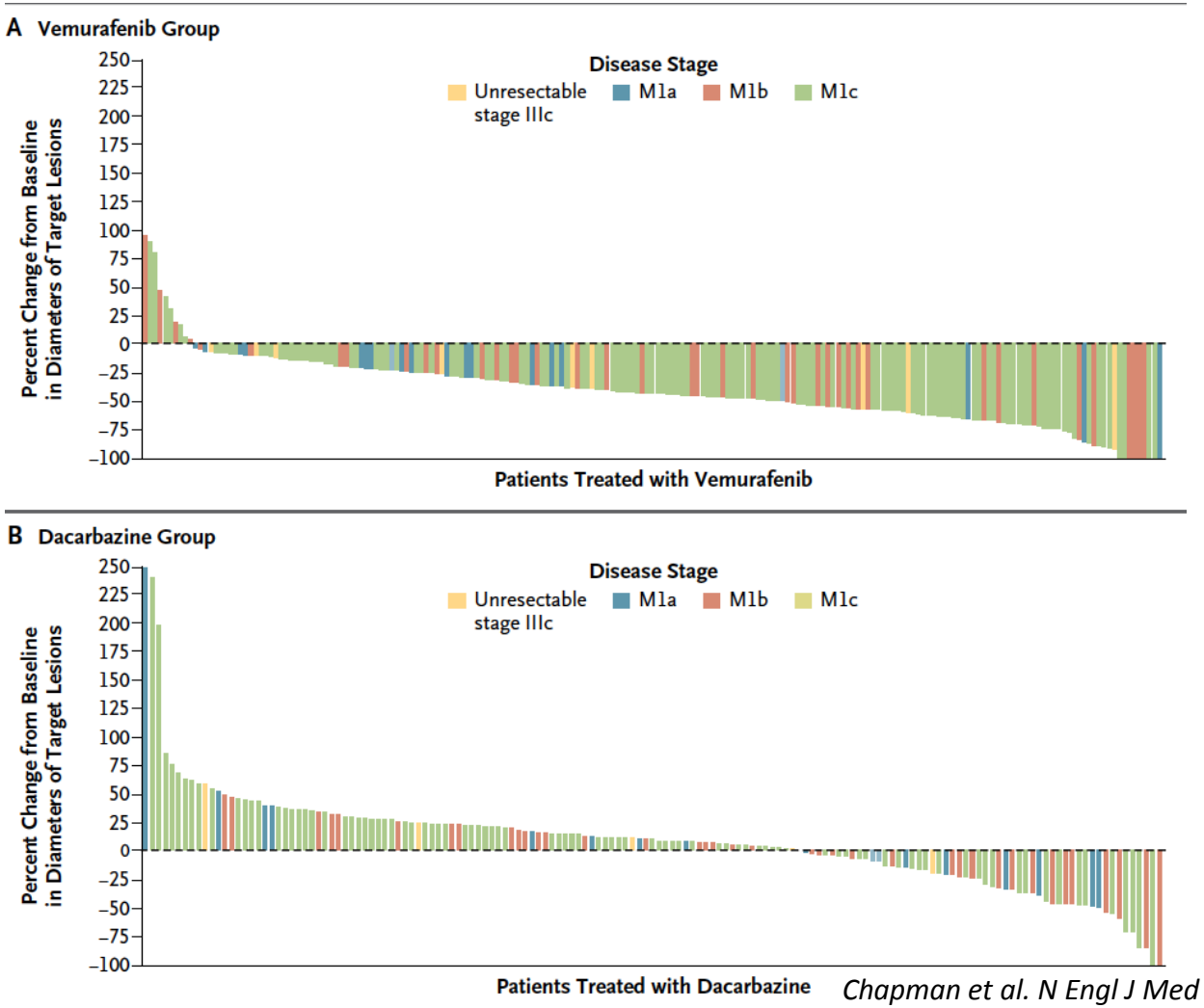
Mutational landscapes can be defined by the most active mutations





Sohrab Shah, Ali Bashashati, Jiarui Ding and team

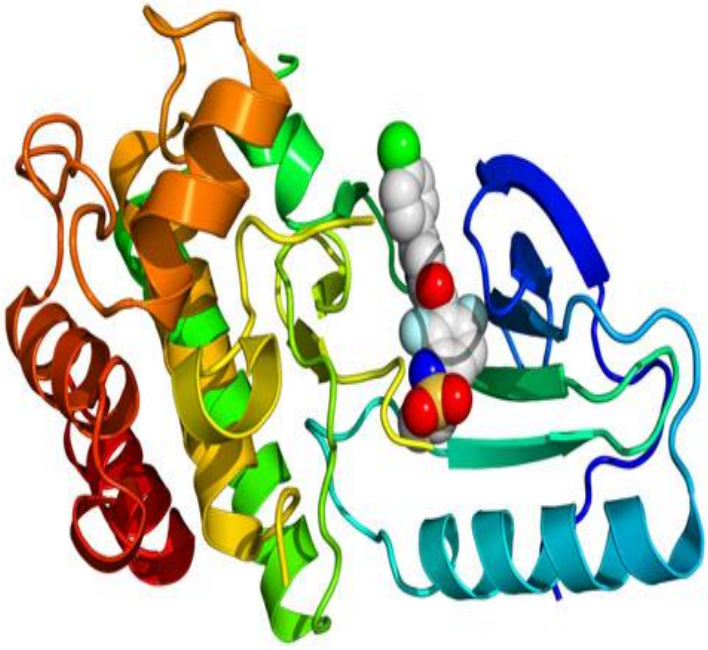
Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation



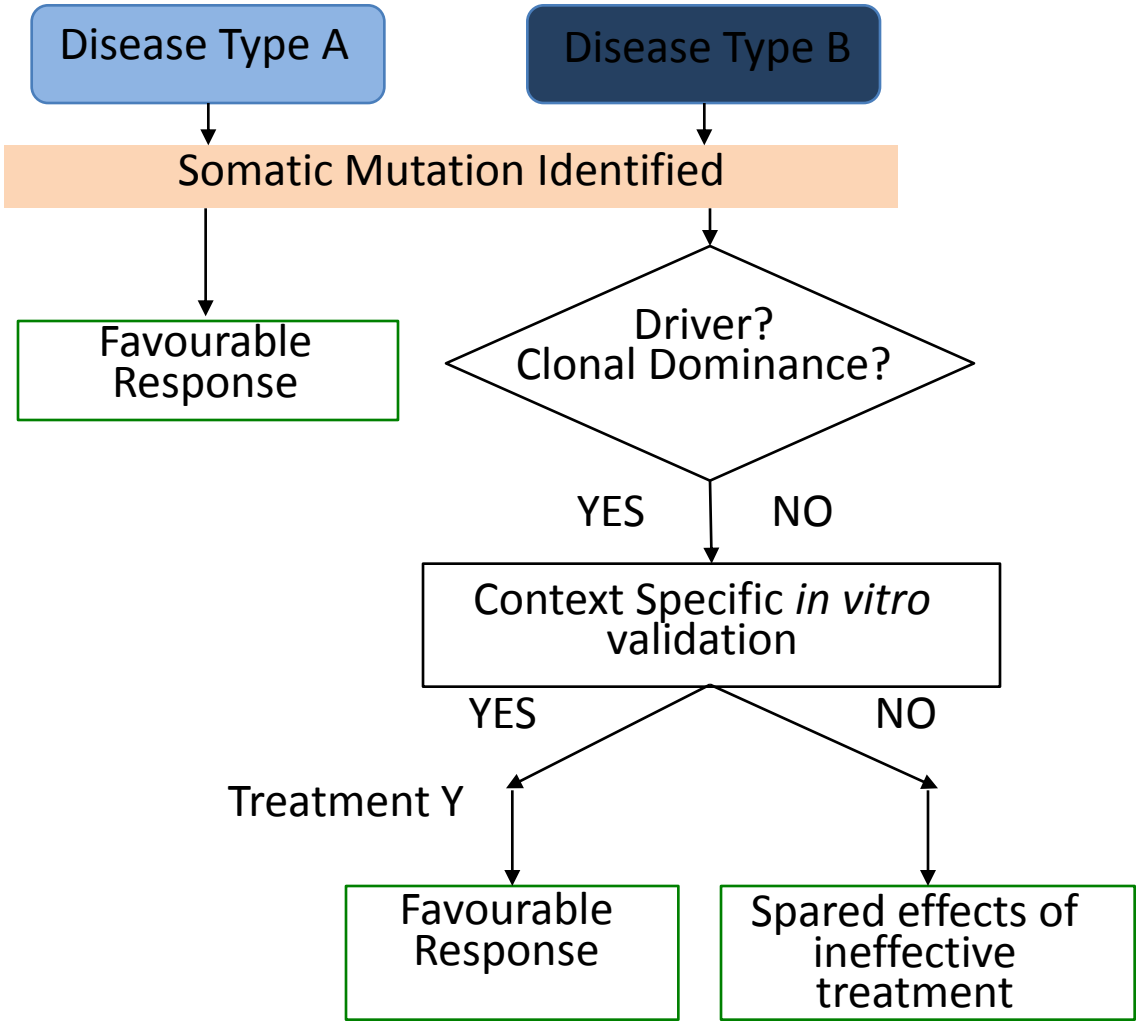
Chapman et al. N Engl J Med 2011;364:2507-16.

Same mutation, same drug so why no response in colon cancer?

- In CRC but not melanoma BRAF inhibition leads to a surge in EGFR expression and pAKT
- Dual treatment could work
- **Prahallad et al Nature 2012**



Contextual Genomics-Based Treatment Stratification



What about relapsed disease ?

- Considering the context of mutations at time zero is not enough – how can we adapt our decisions over time to manage the emergence of resistant disease?

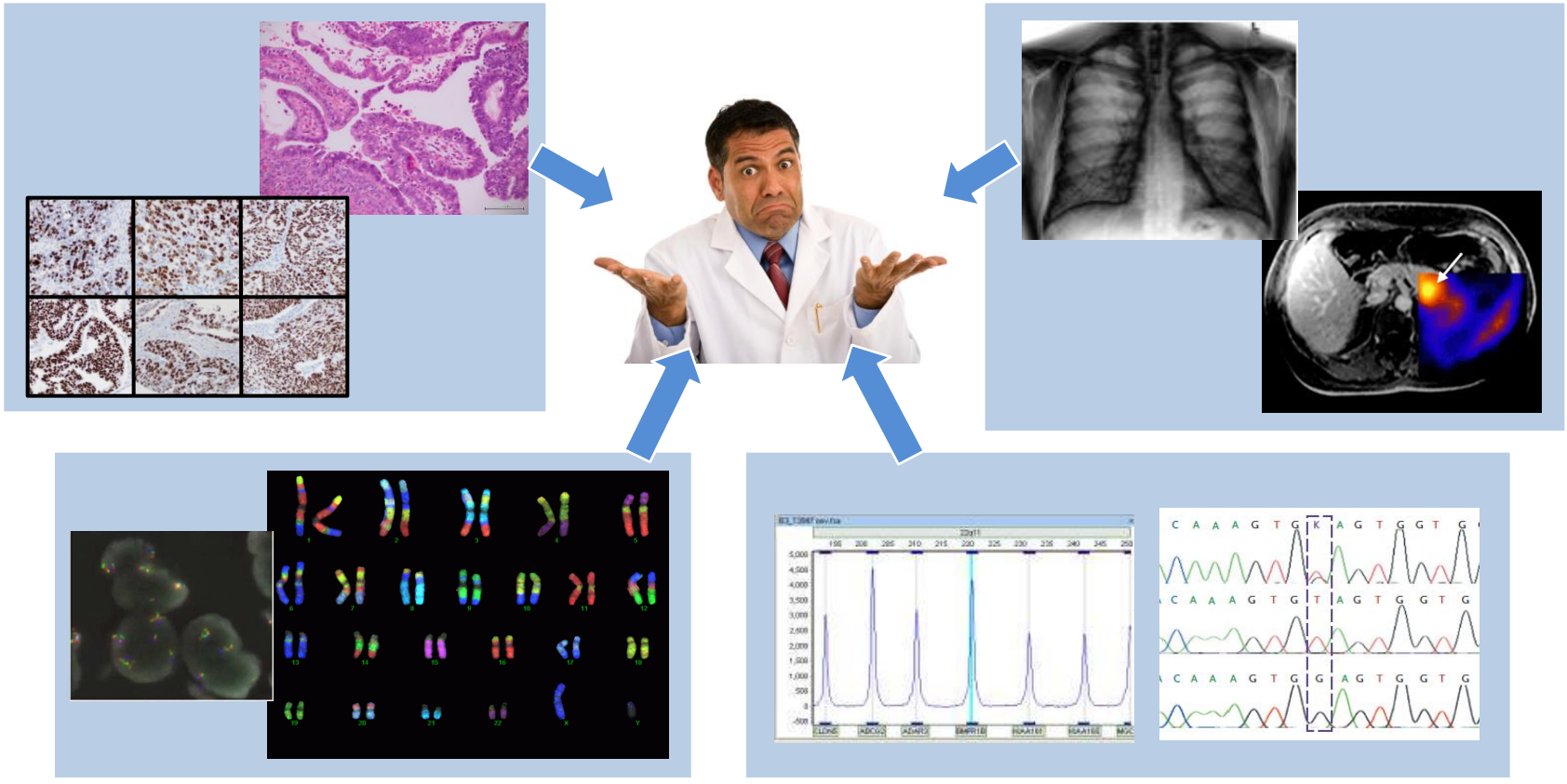
- Considering that single biopsies may not provide information relevant for the whole tumor - is there a better way of sampling cancers for relevant mutations?

1950's Diagnostics

- Kindly physician compiles and interprets data data for admiring patient

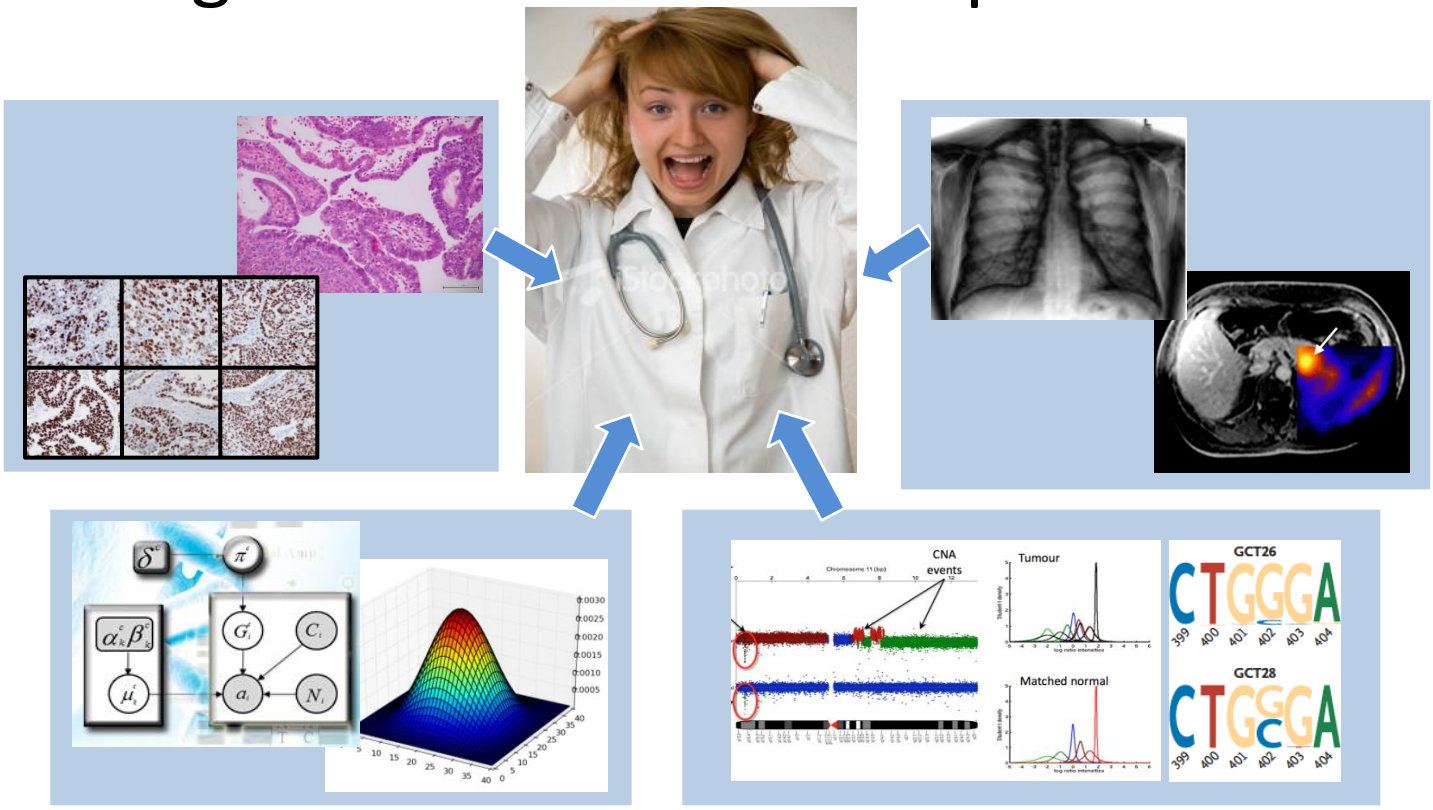
Today's Diagnostics

- There is a little too much data for effective on the fly integration

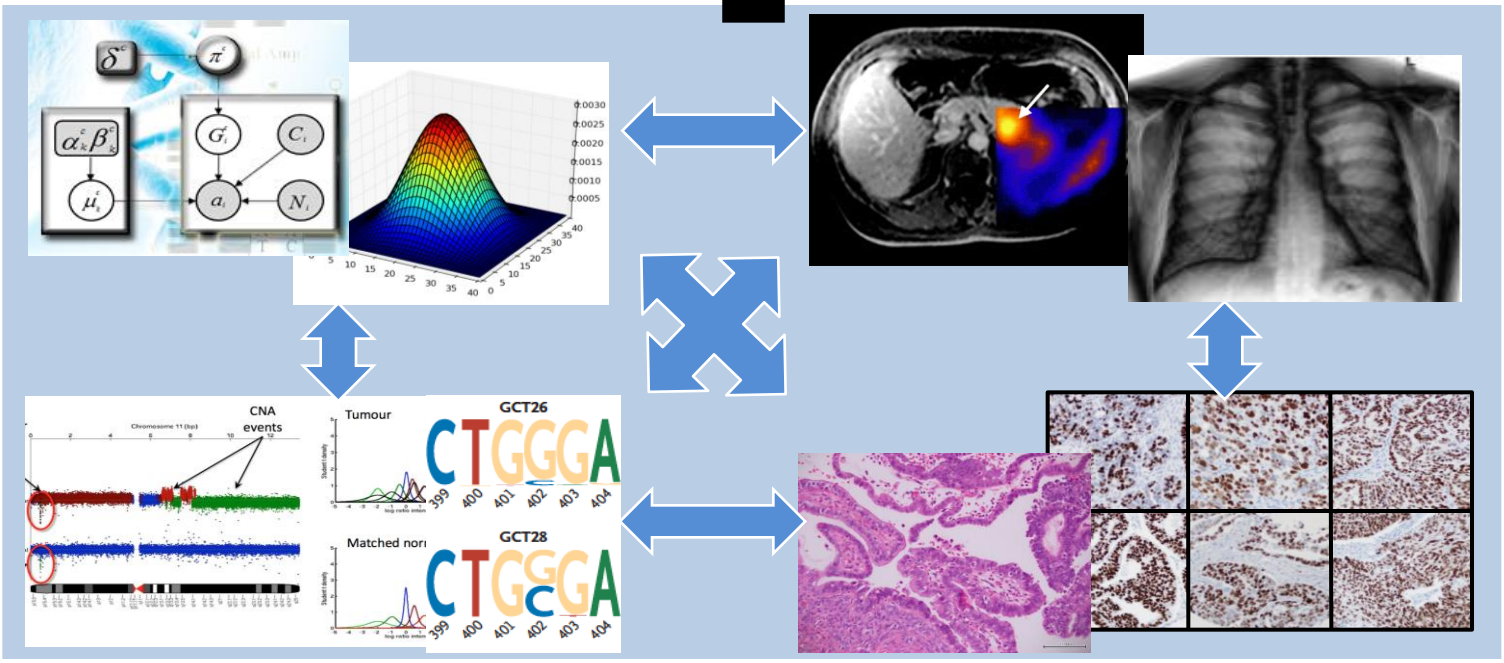


Tomorrow's Diagnostics

- As genomics and bioinformatics become commonplace as decision support tools integration of all diagnostic data will be impossible in the clinic



A Priori integration of diagnostics may optimize utility of genomics in the clinic



Ready to hit the runway- definition #1
Today's fashion- by nature transient and disposable

Ready to hit the Runway Definition #2
Ready to take off- a meaningful departure from the status quo

But if we are rash in our approach to personalizing cancer control

The 200-ton Spruce Goose flies just above the water off Long Beach on Nov. 2, 1947. The flight, the plane's only one, lasted about a minute.