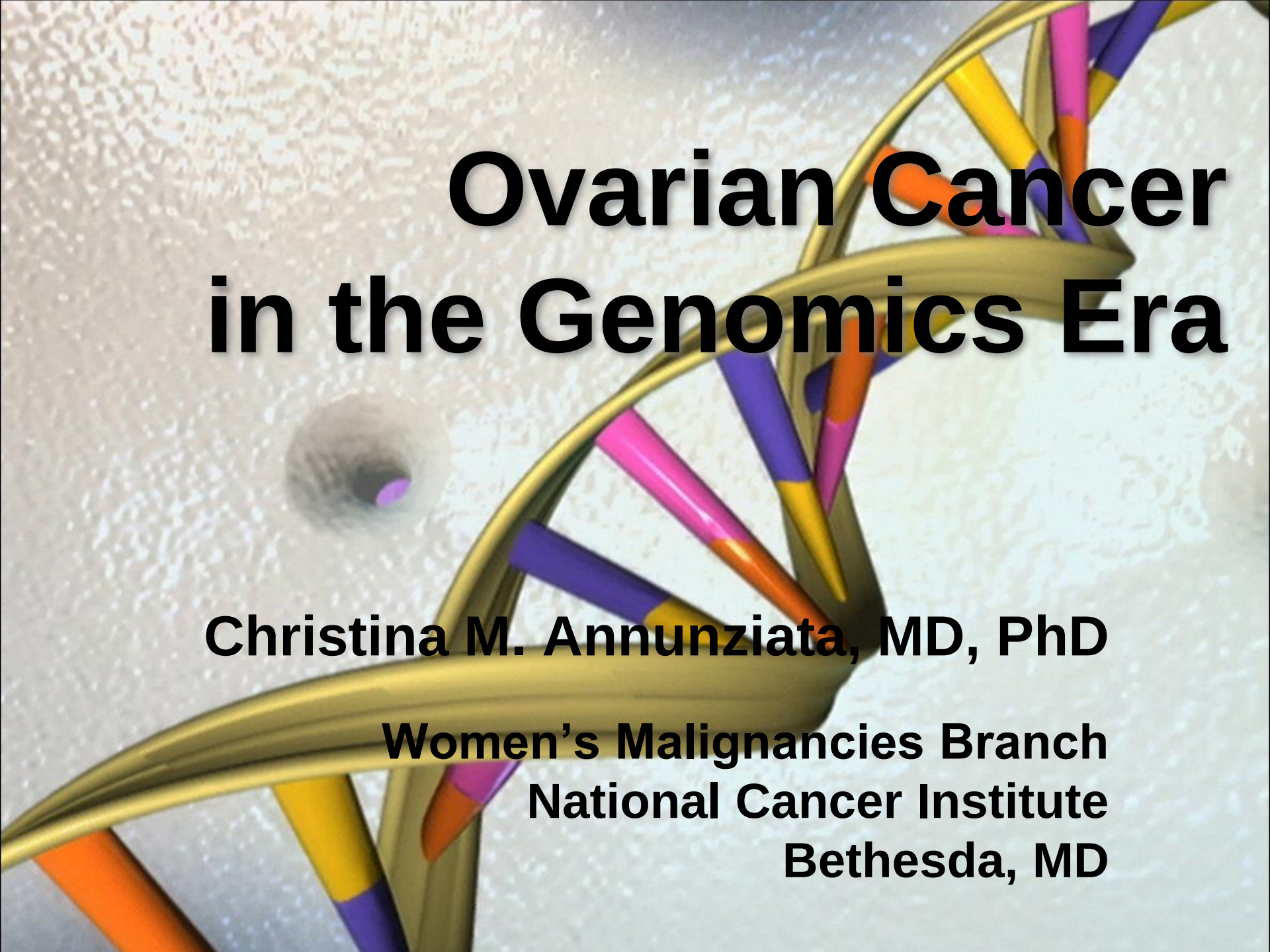


Ovarian Cancer in the Genomics Era

Christina M. Annunziata, MD, PhD

Women's Malignancies Branch
National Cancer Institute
Bethesda, MD

Cancer Genomics

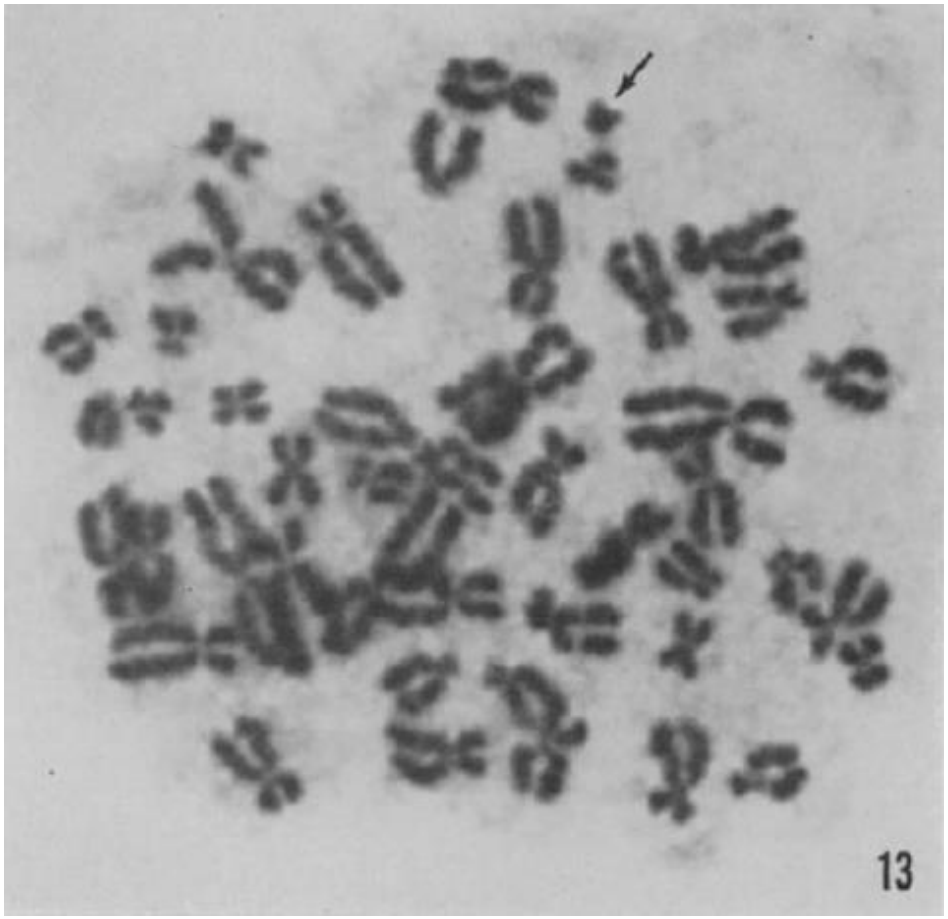
- Study of the genome
  - Chromosomes
  - Gene expression
  - Global analysis (not individual entities)

The Genomics Era

- 1959 – Nowell and Hungerford
  - Study of chromosomes
  - Identified recurrent abnormality
  - Philadelphia chromosome
  - Chronic leukemia

The Genomics Era

- 1959 – Nowell and Hungerford



Genomics Era

The Genomics Era

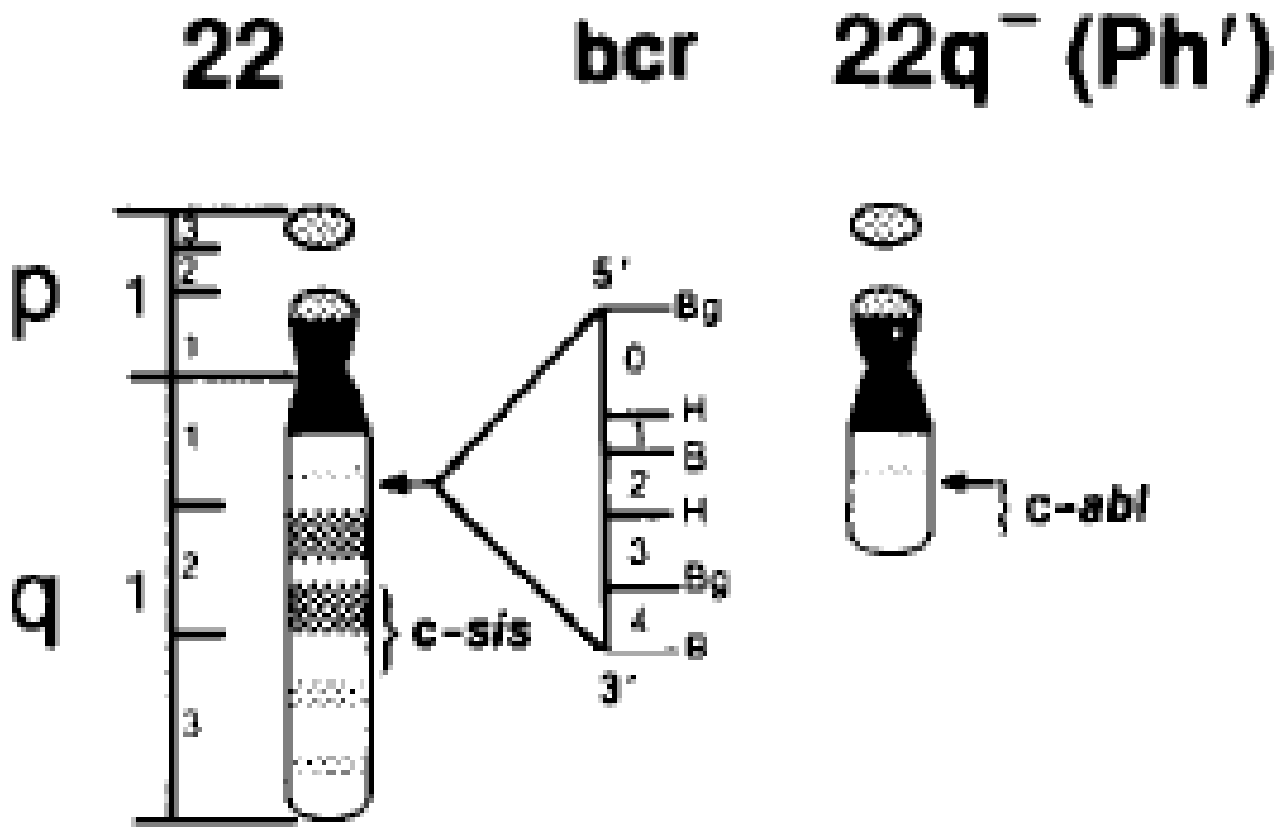
- 1973 – Janet Rowley

Table 1 Summary of Chromosomal Analysis

Case	Age (yr)	Duration of CML (yr)	Karyotype[*6,7]
1§	72	6	46,XY,9q+,22q−
2§	29	3½	48,XY,9q+,+C,+mar,−17,+?F,22q−
3§	37	3½	46,XY,9q+,22q−
			50,XY,9q+,+8,+C,+mar,22q−, +22q−
			50,XY,9q+,+8,+C,+mar,22q−, +22q−
4§	71	1½	46,XX,9q+,+mar,−17,22q−
			47,XX,9q+,+C,+mar,−17,22q−
5§‡	51	2½	48,XY,9q+,+mar,22q−,+22q−
6	45	2 mo	46,XX,9q+,22q−
7	25	1	46,XX,9q+,22q−
8	18	3	46,XX,9q+,22q−
9	64	3½	46,XX,9q+,22q−

The Genomics Era

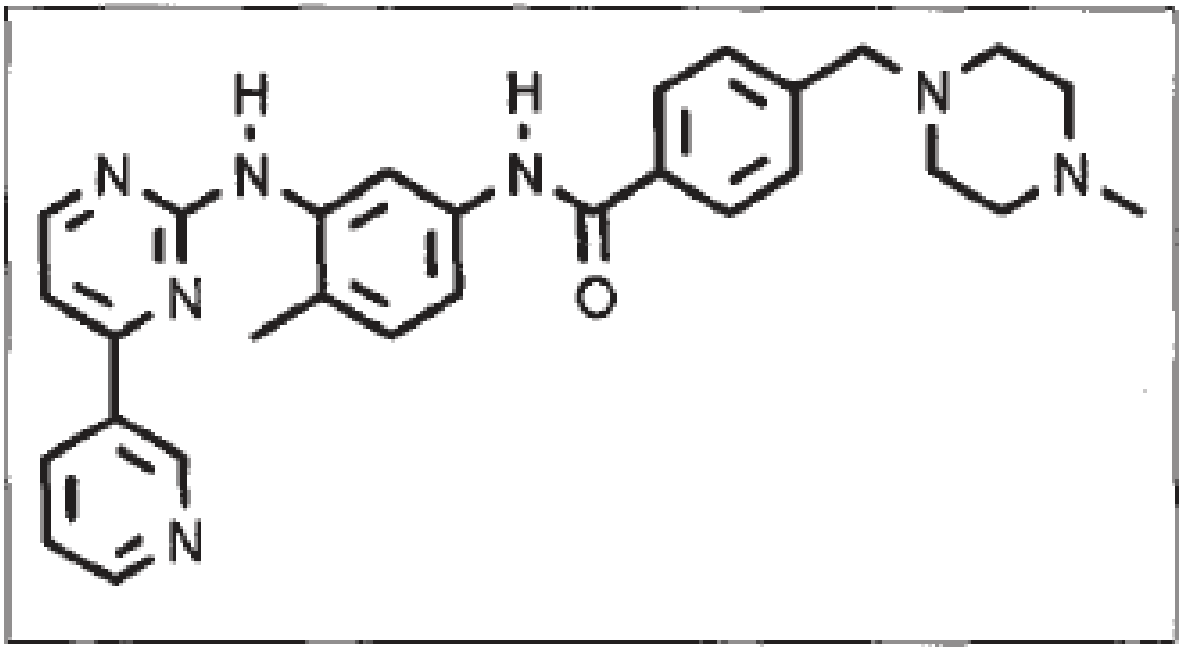
- 1984 – Groffen – BCR-ABL



The Genomics Era

- 1996 – Drucker – blocking ABL

Fig. 1 Structure of CGP 57148.



Functional Genomics

- What part of the genome is functional
- Causes an effect
- Transforms normal cells into cancer
- Looking for “driver” alterations

Functional Genomics

- 1981 – Shih – discovery of Her2/neu

Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts

Chiaho Shih, L. C. Padhy, Mark Murray & Robert A. Weinberg

Department of Biology and Center for Cancer Research

Functional Genomics

- 1984 – Schechter – neu and EGFR

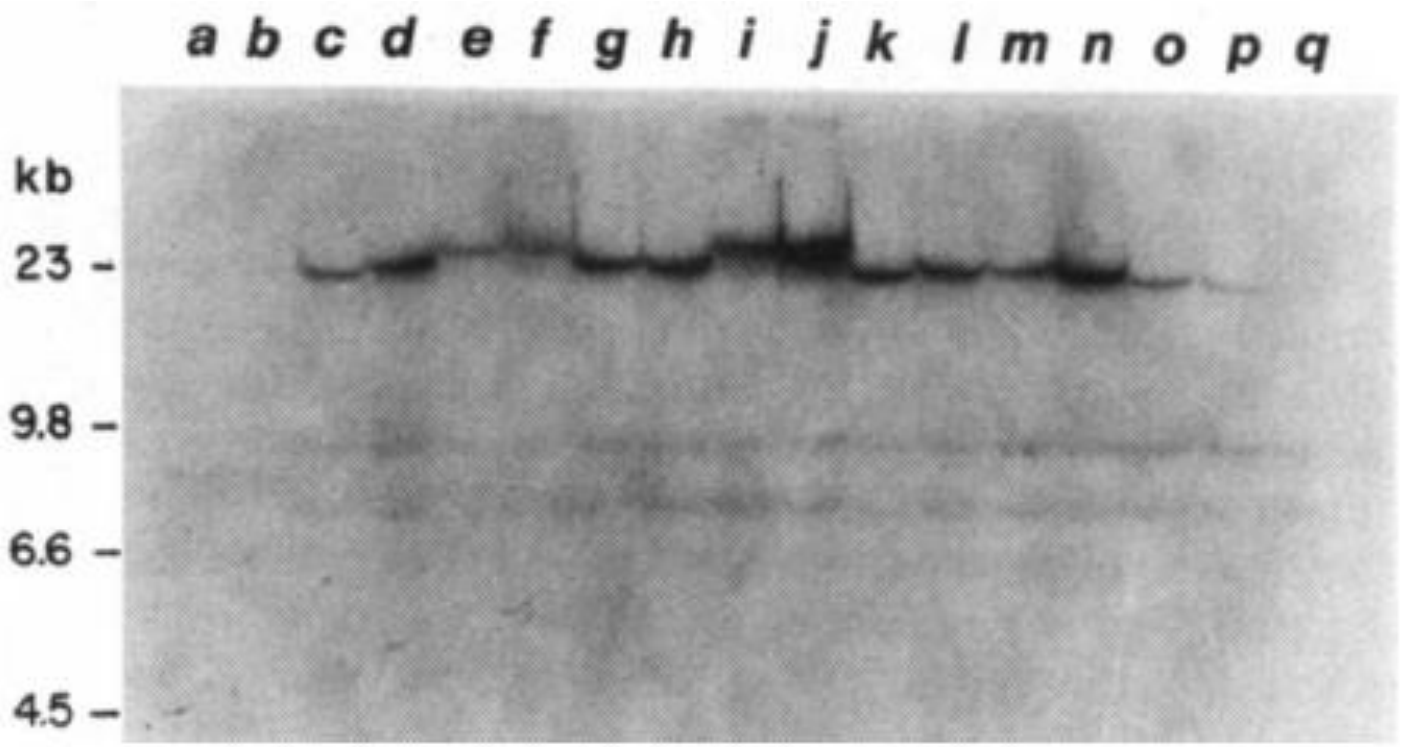
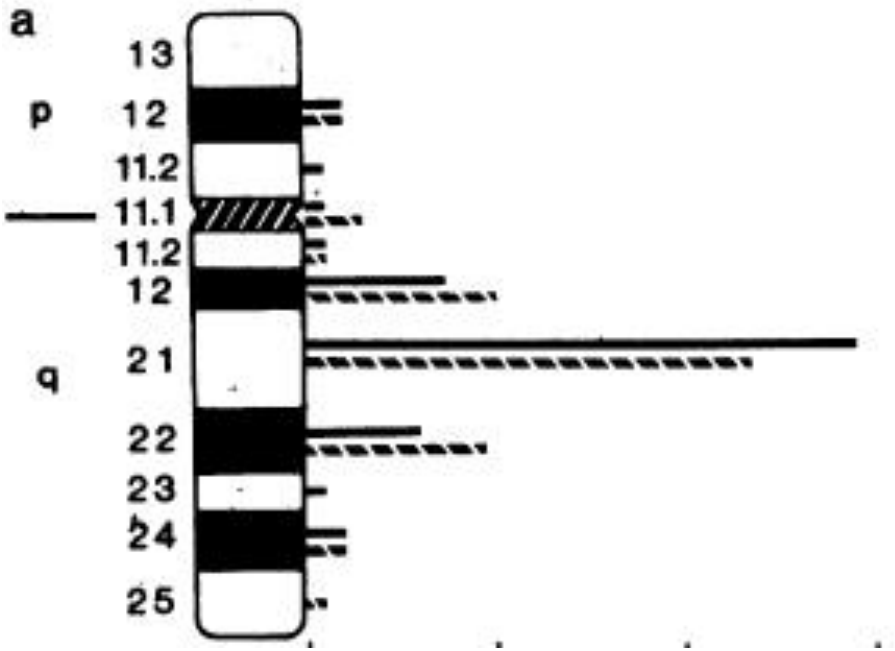
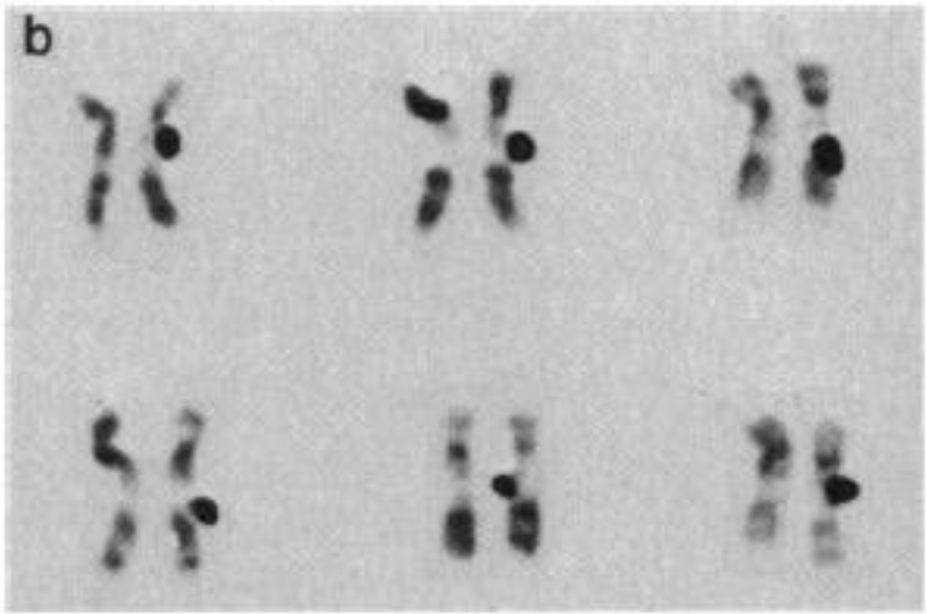


Fig. 1 Southern blot analysis of *erb-B*-related sequences in NIH 3T3 cells transformed with rat neuro/glioblastoma DNAs;

Functional Genomics

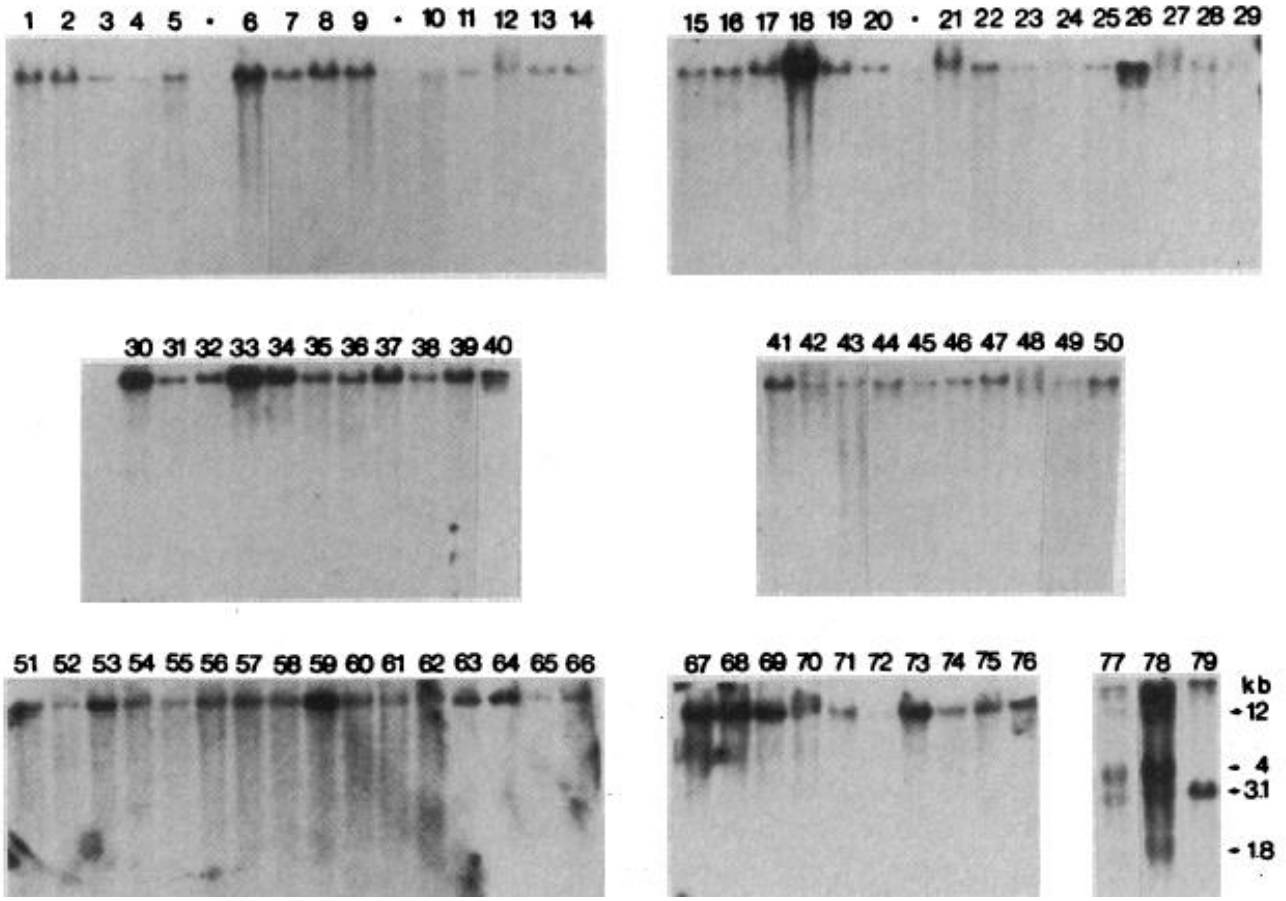
- 1985 – Coussens – Her2 on chromosome 17





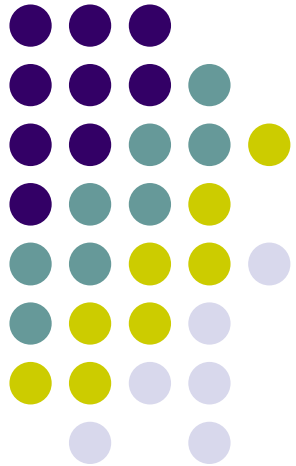
Functional Genomics

- 1987 – Slamon – HER2 in breast cancer

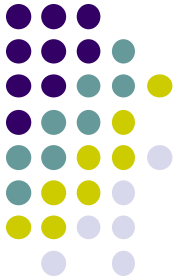


Using genomics to study ovarian cancer

Do we have any “drivers”?



Ovarian Cancer



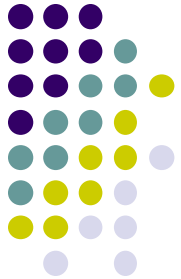
- Most lethal gynecologic malignancy in the US
  - >16,000 deaths/yr
  - 5th most common cancer death for women
- 70% diagnosed with advanced disease
- < 35% of advanced stage patients alive at 5y

Ovarian Cancer Stages

Ovarian Cancer

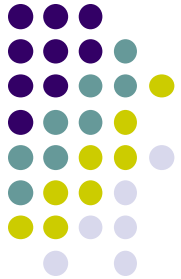
Stage	Description	Incidence	Survival
I	Confined to ovaries	20%	90%
II	Confined to pelvis	5%	65%
III	Spread IP or nodes	58%	45%
IV	Distant metastases	17%	<5%

Treatment for Newly Diagnosed Ovarian Cancer



- Complete surgical staging
- Optimal reductive surgery
- Chemotherapy
- *Clinical Trials*

The State of Treatment for Newly Diagnosed Ovarian Cancer



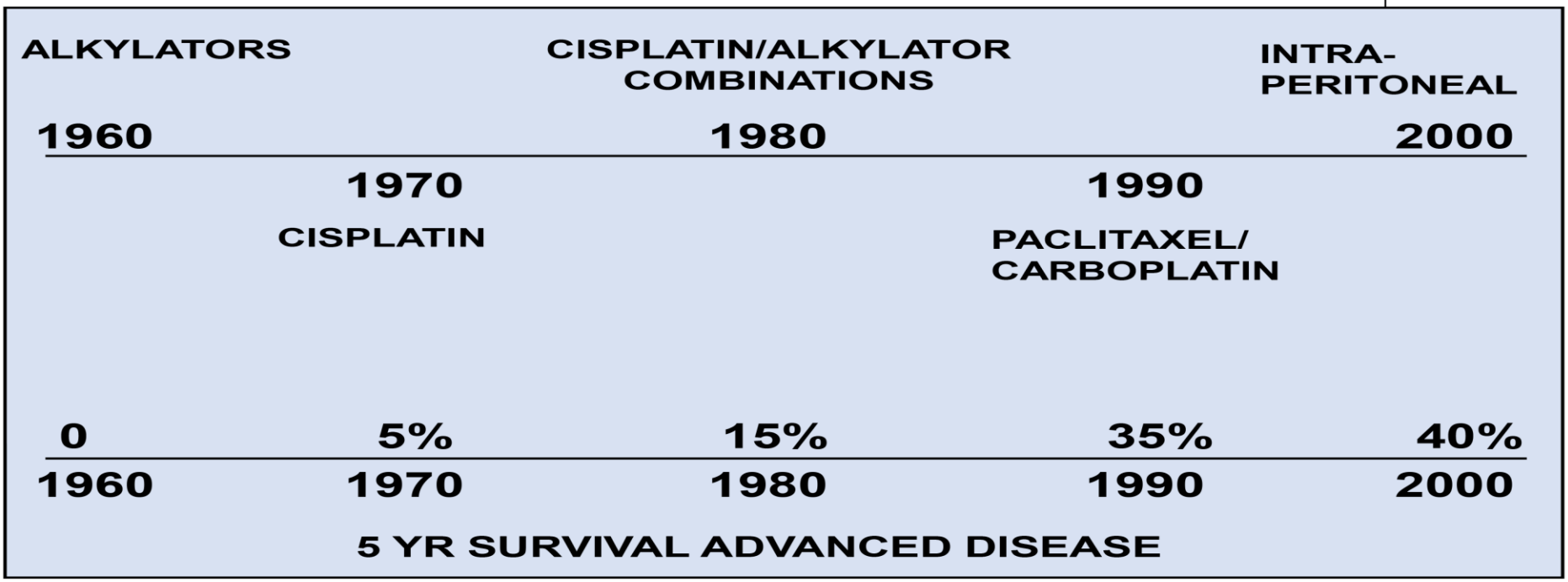
- Complete surgical staging
- Optimal reductive surgery
- **Chemotherapy**
  - Platinum = cisplatin or carboplatin

    AND
  - Taxane = paclitaxel or docetaxel
  - *Intraperitoneal if Stage III, optimal reduction*

- *Clinical Trials*

Treatment and Outcome

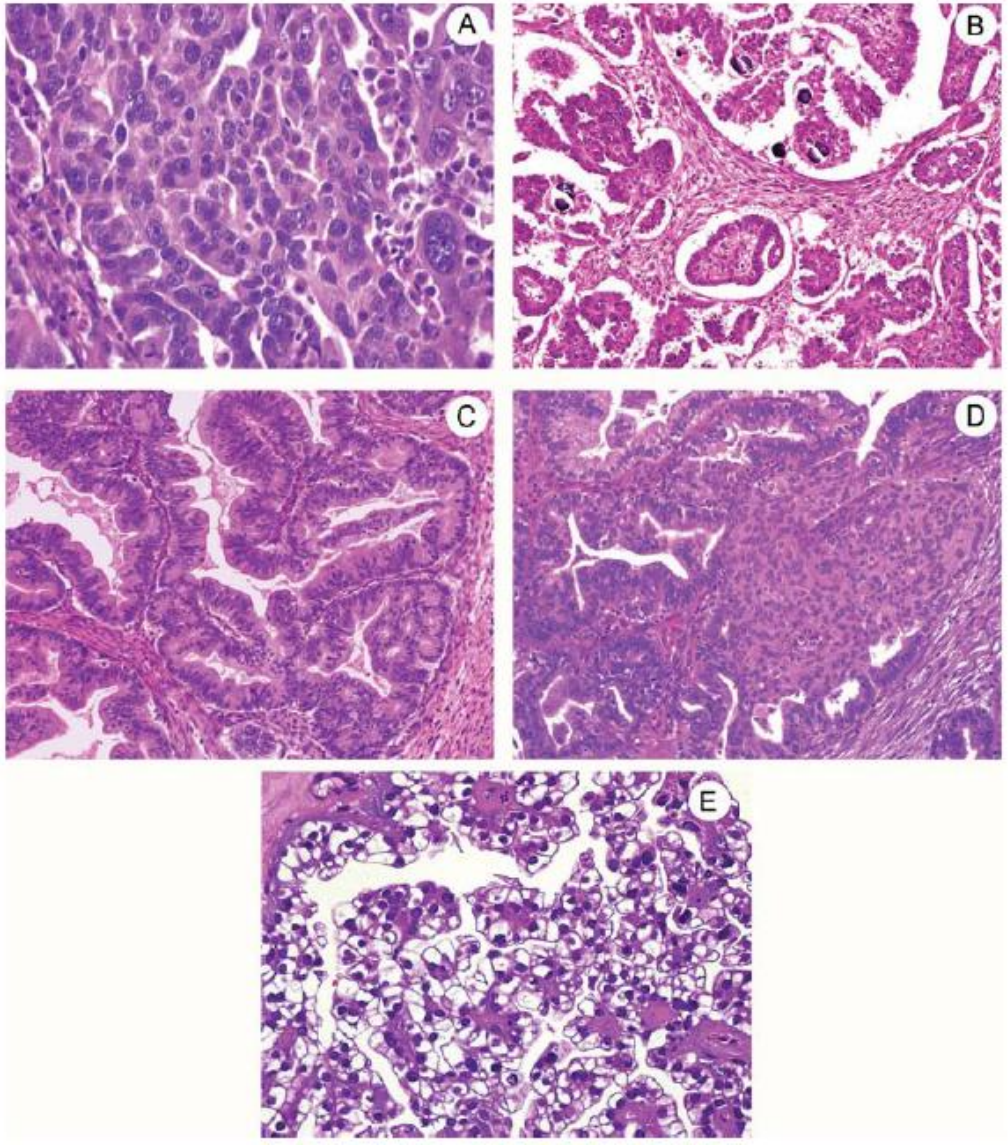
Treatment and Outcome for Advanced Ovarian Cancer



Ovarian Cancer

Prevalence

- Serous – 80%
- Endometrioid – 10%
- Clear cell – 5%
- Mucinous – 3%
- Other – 2%



Ovarian Cancer

Ovarian Cancer

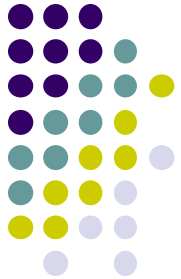
Prevalence

- Serous – 80%
- Endometrioid – 10%
- Clear cell – 5%
- Mucinous – 3%
- Other – 2%

Tissue of origin

- Fallopian tube?
  - Serous
- Endometriosis?
  - Endometrioid and clear cell
- Mullerian epithelium
  - Extra-uterine

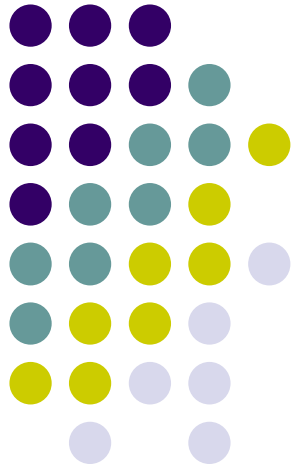
Ovarian Cancer



- Increasing our understanding about the biological and biochemical events underlying ovarian cancer progression will create avenues for new treatments

- Can we use Genomics?

Clear cell, Endometrioid



Clear Cell cancers

- 5-10% of all cases (serous = 70%)
- Worse response to standard chemotherapy
- Associated with endometriosis (up to 40%)

Clear cell ovarian cancer

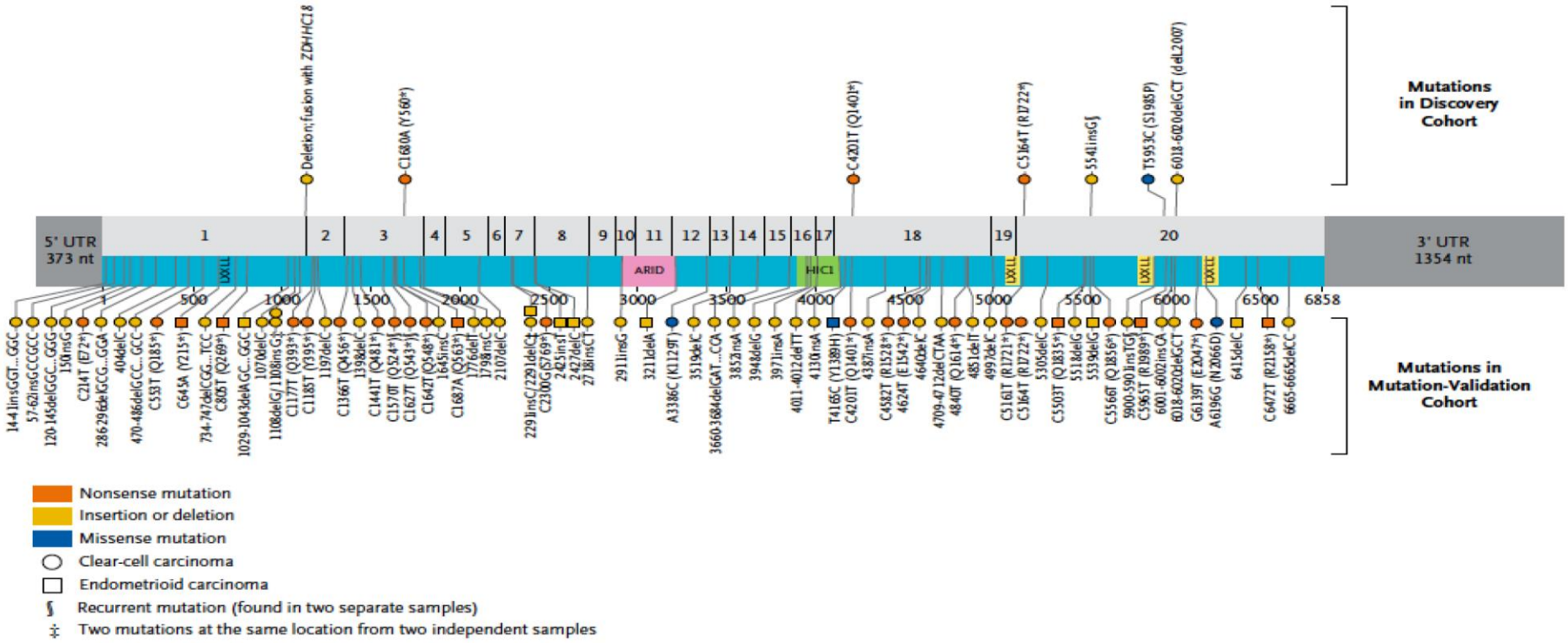
Clear cell OC – genomics

- Sequenced RNA from 18 clear cell ovarian cancers, and one cell line (discovery)
- Sequenced DNA exons from 210 samples
  - 101 more clear cell, 33 endometrioid, 76 serous, 1 more clear cell line (validation)
- Immunostain 455 more samples
  - 132 clear cell, 125 endometrioid, 198 serous

Weigand, *NEJM* 2010

ARID1A mutations in clear cell

ARID1A mutations in clear cell

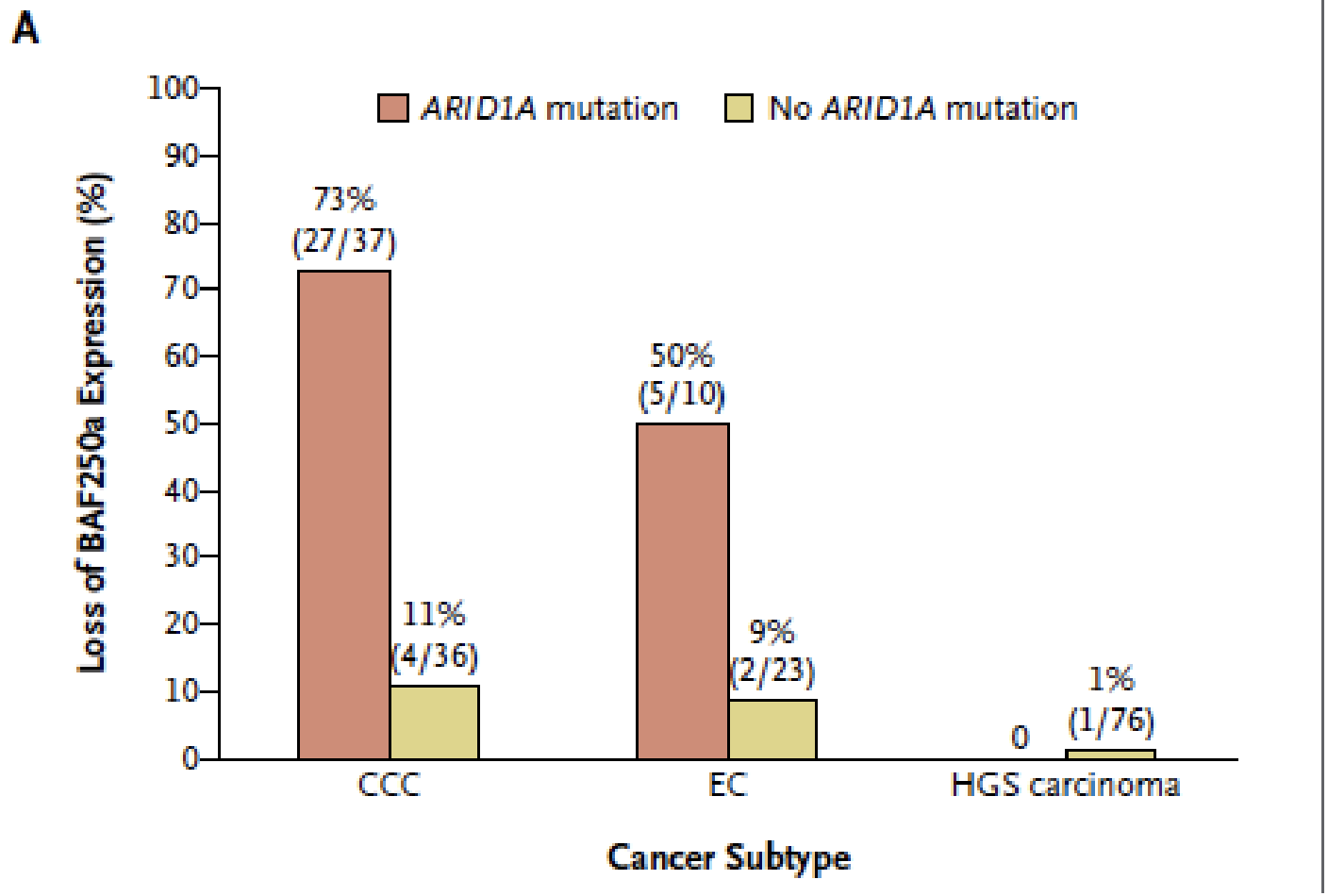


Weigand, *NEJM* 2010

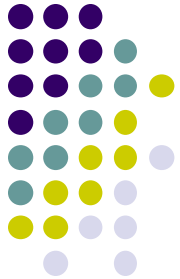
ARID1A

- SWI-SNF chromatin remodeling complex
- Mutated in breast cancer, lung cancer
- 1p36: deleted 6% of all cancers
- Tumor suppressor gene?

ARID1A mutations

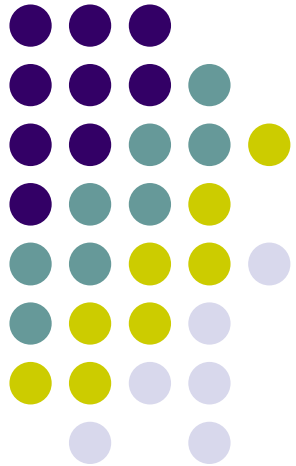


Clear cell and endometrioid cancer

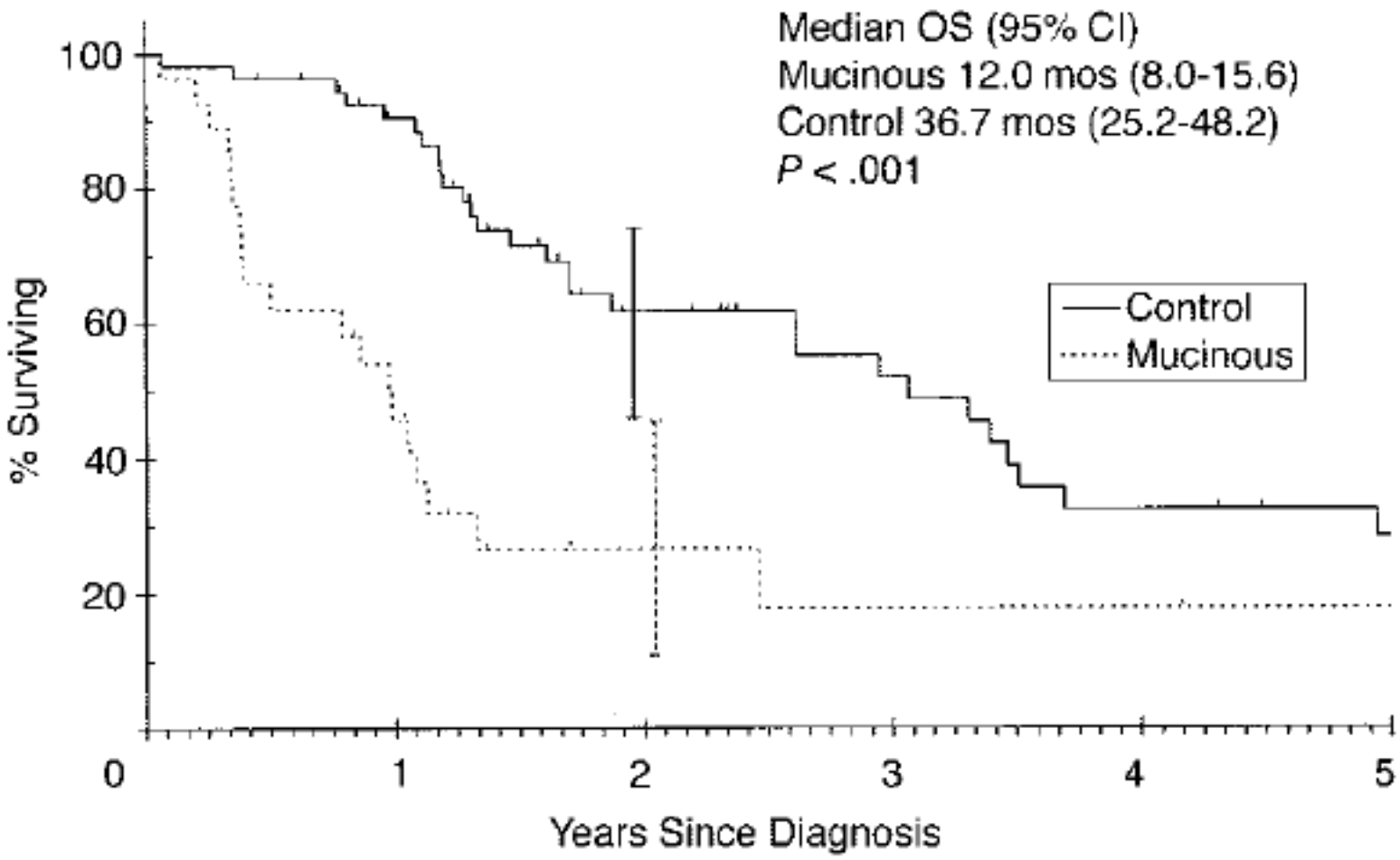


- ARID1A mutated or lost in
  - Over 40% clear cell
  - 30% endometrioid
  - Less than 1% serous
- Unknown oncogenic mechanism
  - No indication of which resulting pathways affected
  - Unclear therapeutic utility
- Diagnostic utility?
  - Not a ‘functional’ experiment

Mucinous

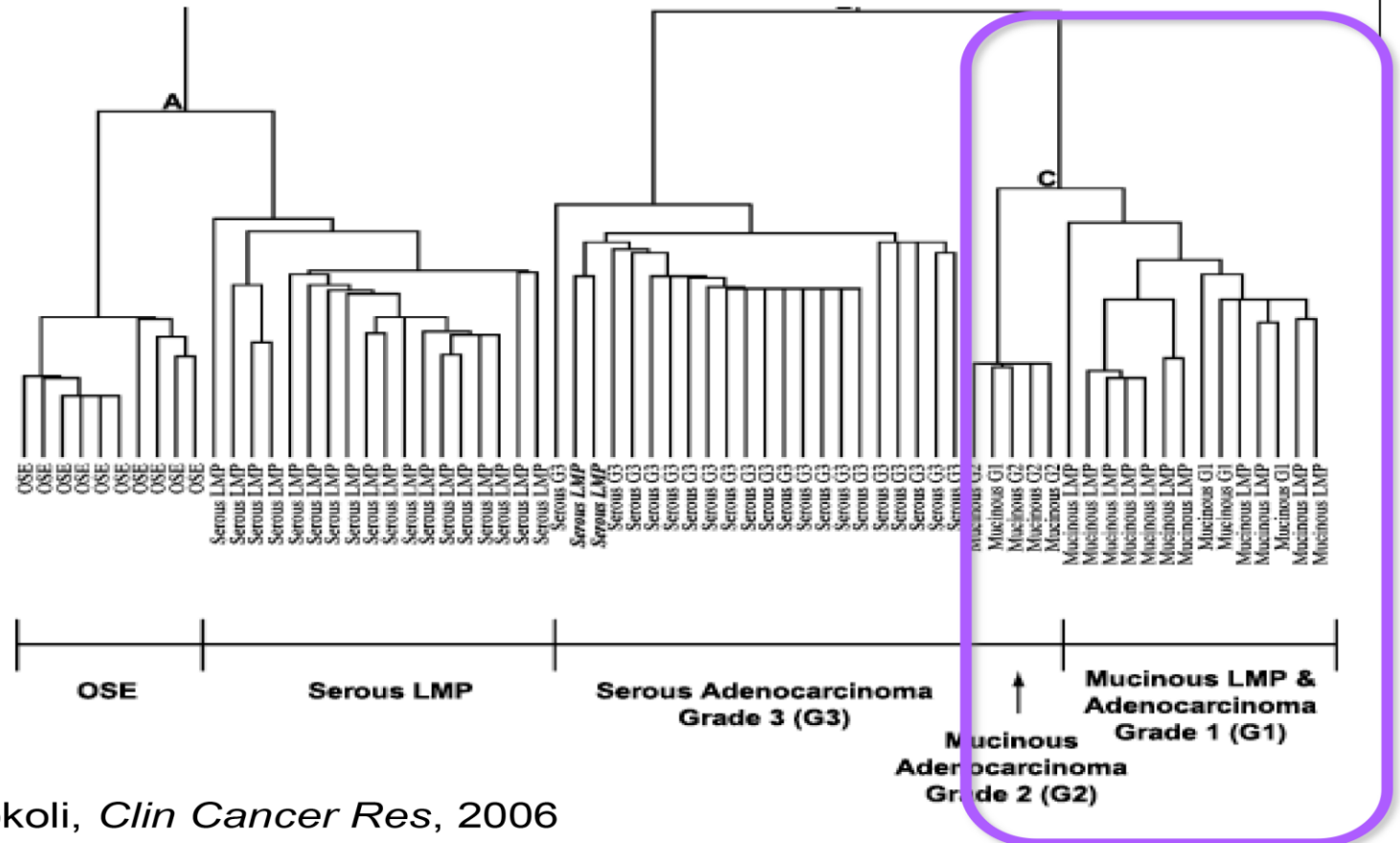


Mucinous ovarian cancer



Gene expression

Gene expression – mucinous versus serous



Wamunyokoli, *Clin Cancer Res*, 2006

K-ras mutations

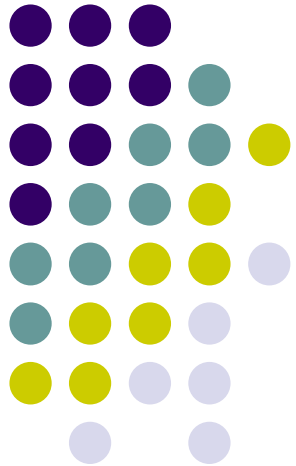
KRAS mutations - mucinous

Table 2: KRAS mutation frequencies observed in borderline malignancies

	borderline		
histotype	**n**	**mutated**	**% mutated**
serous	20	7	35.00
endometroid	1	0	0.00
mucinous	6	3	50.00
unknown	2	0	0.00
total	29	10	34.48

Auner, *BMC Cancer* 2009

Low grade serous



KRAS and BRAF mutations

KRAS and BRAF mutations

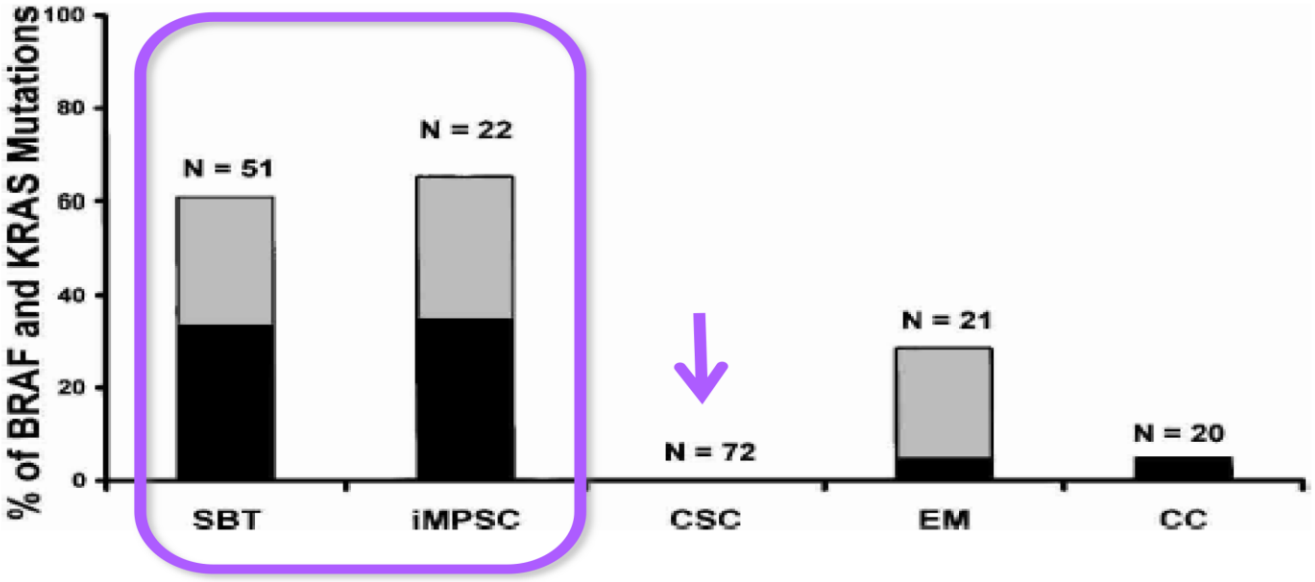
- BRAF codon 599
- KRAS codon 12 or 13

- 15 of 22 (68%) of low grade serous cancers
- 31 of 51 (61%) precursor lesions (SBT)
- None of 72 high grade serous cancers

Singer, *JNCI* 2003

KRAS and BRAF

KRAS and BRAF mutations



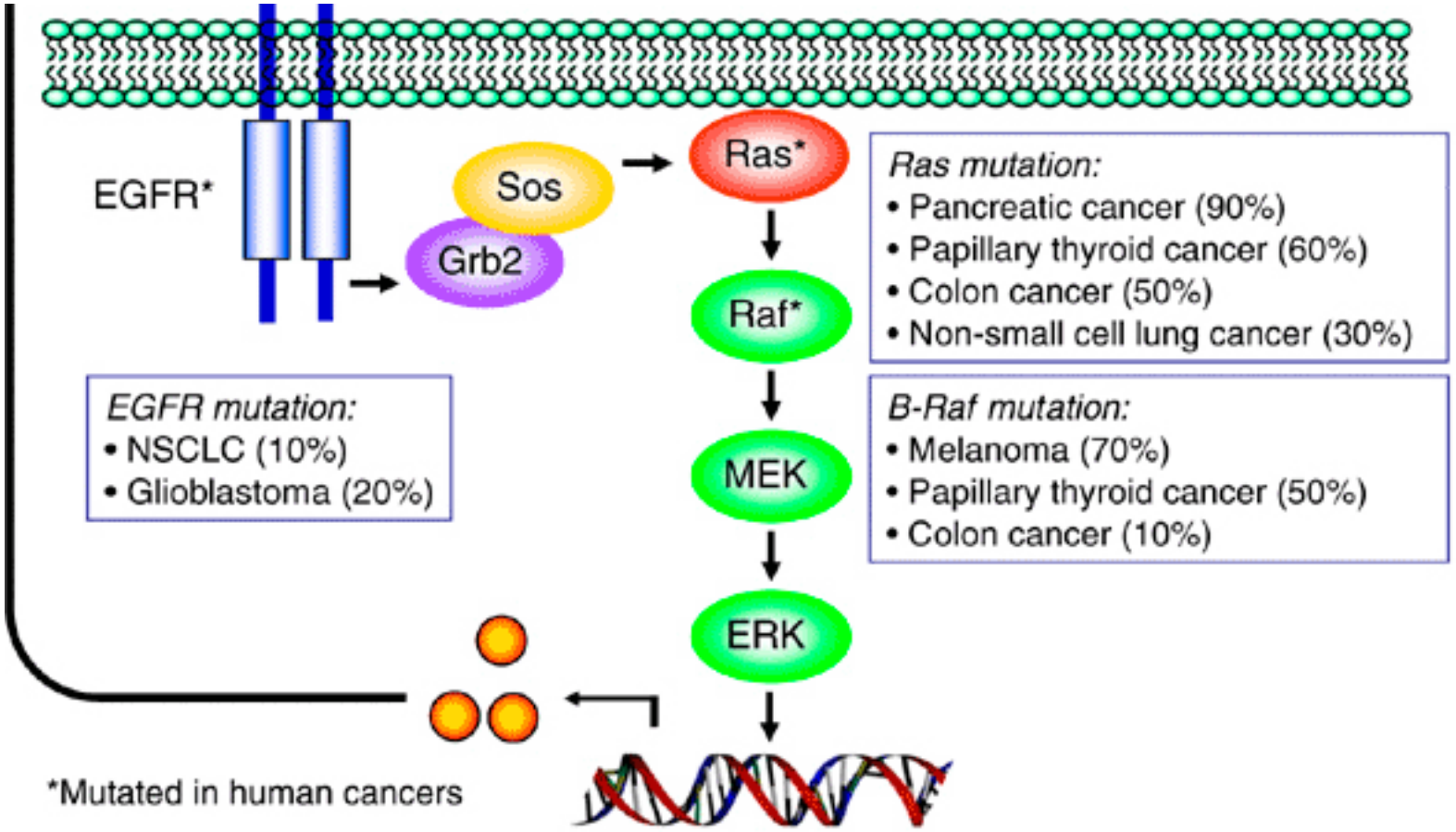
Serous borderline tumors

Invasive low grade serous cancers

High grade serous cancers

Singer, *JNCI* 2003

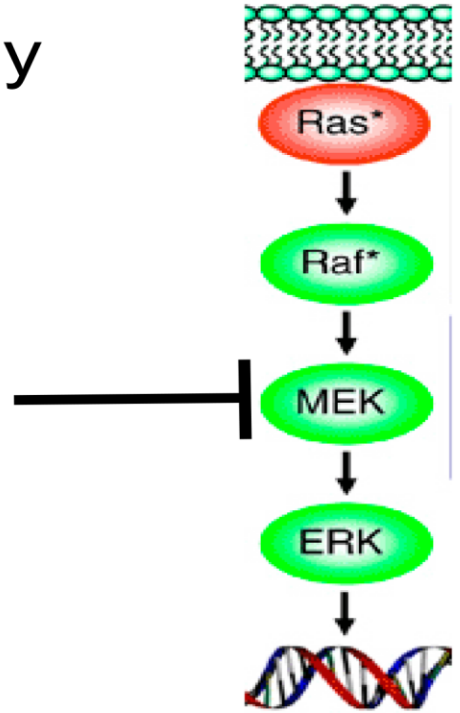
RAS signaling pathway
- a potential driver?



MEK inhibitor

Clinical trial: MEK inhibitor

- Recurrent Low Grade Serous ovarian cancer
- Selumetinib 50 mg twice daily
- 52 patients
  - 8 responses
  - 34 stable disease >4mo



Farley, *Lancet Oncol* 2013

Selumetinib responses

	Number	No tumour response	Tumour response	p value*
Total	34	27 (79%)	7 (21%)	
BRAF mutation				
No	32	25 (78%)	7 (22%)	1·000
Yes	2	2 (100%)	0	
KRAS mutation				
No	20	15 (75%)	5 (25%)	0·672
Yes	14	12 (86%)	2 (14%)	
BRAF or *KRAS* mutation				
No	18	13 (72%)	5 (28%)	0·405
Yes	16	14 (88%)	2 (13%)	

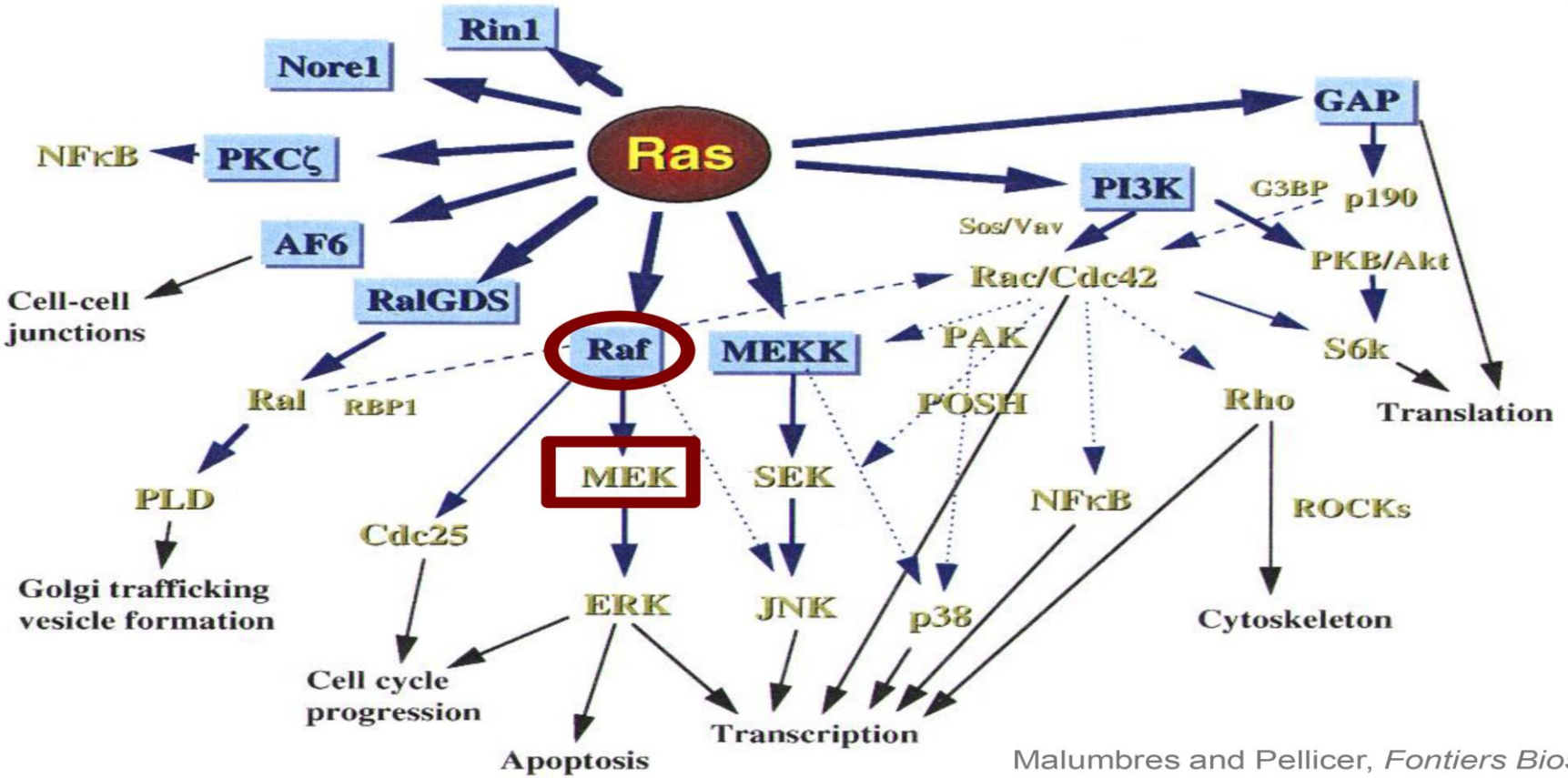
Data are number (%), unless otherwise indicated. *Fisher's exact test.

Table 8: **Tumour response (complete or partial) by *BRAF* and *KRAS* mutations**

Farley, *Lancet Oncol* 2013

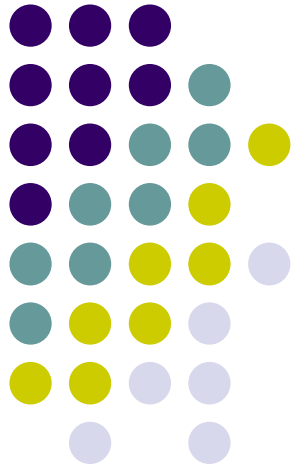
RAS signaling

RAS signaling



Malumbres and Pellicer, *Fontiers Biosci* 1998

High grade serous



High grade serous cancers

High grade serous cancers

- **The Cancer Genome Atlas (TCGA)**
  - Clinically annotated HGS-OvCa samples
  - Identify molecular abnormalities that
    - influence pathophysiology,
    - affect outcome and
    - constitute therapeutic targets.
  - Microarray analyses: 489 HGS-OvCa tumours,
    - mRNA expression,
    - microRNA (miRNA) expression,
    - DNA copy number and
    - DNA promoter methylation for and
  - Whole exome DNA sequence: 316 samples.

The Cancer Genome Atlas, *Nature* 2011

High grade serous cancers

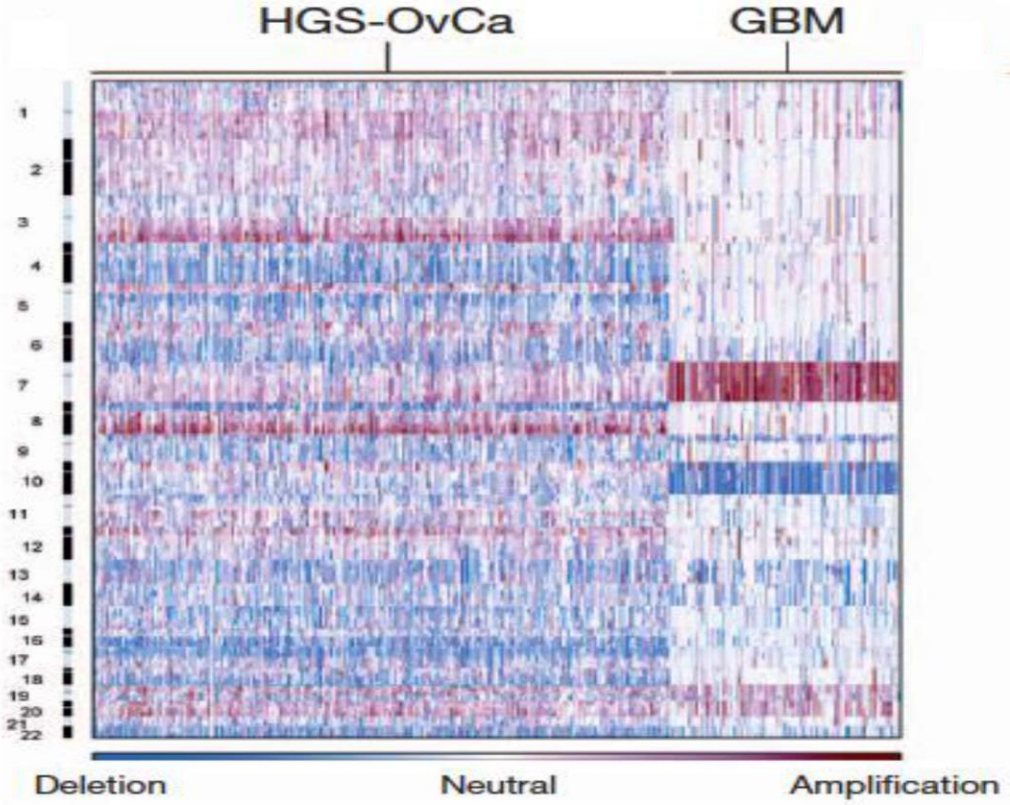
- **Sample inclusion criteria**
  - Newly diagnosed patients
  - ovarian serous adenocarcinoma
  - no prior treatment
  - companion normal tissue specimen
    - adjacent normal tissue,
    - peripheral lymphocytes,
    - or previously extracted germline DNA

Genome copy number

Genome copy number abnormality

Copy number profiles of 489 HGS-OvCa, compared with profiles of 197 glioblastoma multiforme (GBM) tumours.

Copy number increases (red) and decreases (blue) are plotted as a function of distance along the normal genome (vertical axis, divided into chromosomes).



Mutated genes

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

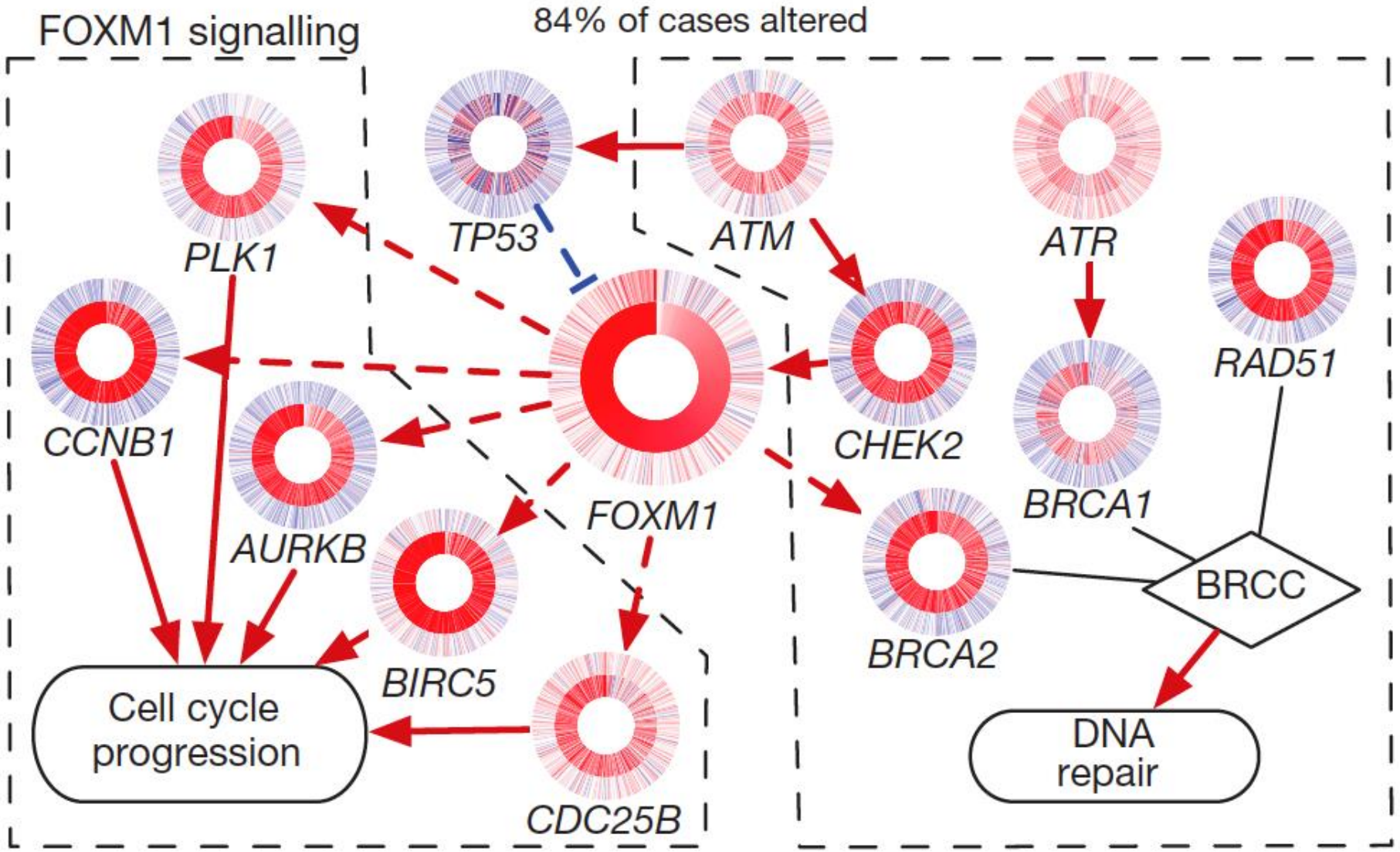
Validated mutations are those that have been confirmed with an independent assay. Most of them are validated using a second independent whole-genome-amplification sample from the same tumour. Unvalidated mutations have not been independently confirmed but have a high likelihood to be true mutations. An extra 25 mutations in *TP53* were observed by hand curation.

The Cancer Genome Atlas, *Nature* 2011

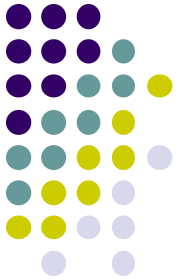
Altered pathways

Altered pathways in HGS-OvCa

Altered pathways in HGS-OvCa



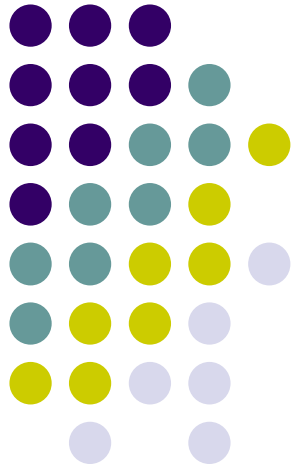
TCGA – what next?



- New **therapeutic** approaches?
  - 50% with HR defects : **PARP inhibitors**
  - commonly deregulated pathways: RB, RAS/PI3K, FOXM1, NOTCH, provide opportunities for therapeutic treatment
  - Inhibitors exist for 22 genes in regions of recurrent amplification
- aberrant genes or **networks**: targeted therapies selected to be effective ...

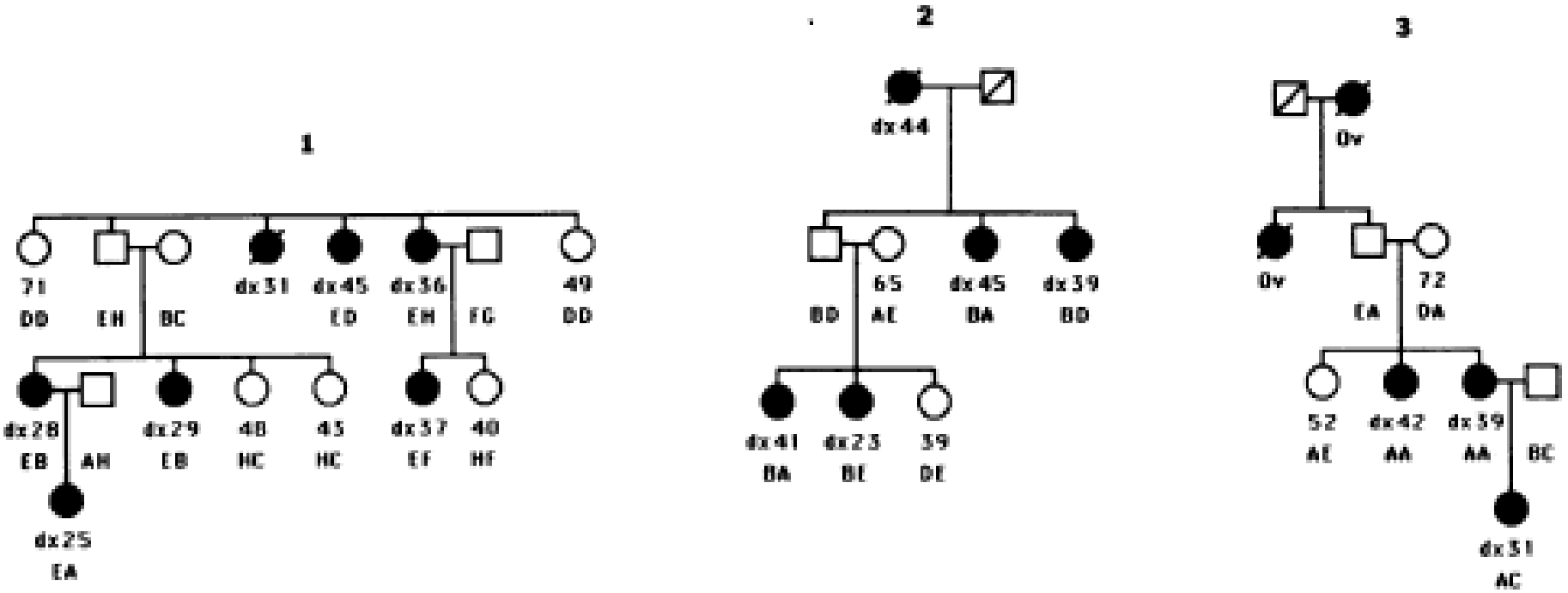
Targeting deficient Homologous Recombination

PARP inhibitors



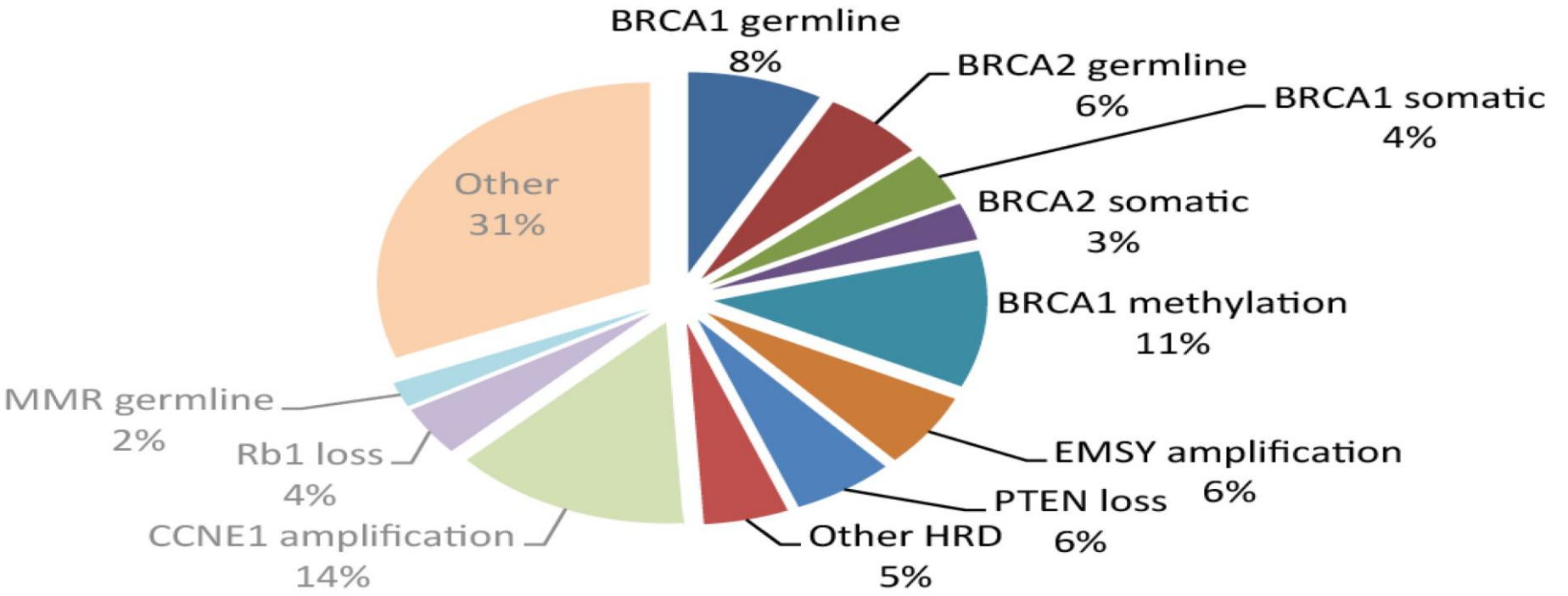
BRCA mutations

- Hall…King, *Science*, 1990



High grade serous cancers

High grade serous cancers

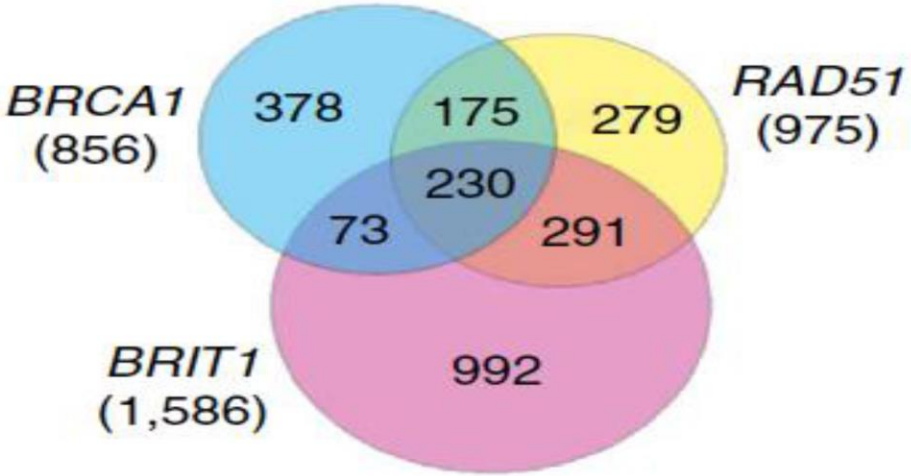


* HRD, homologous recombination defect

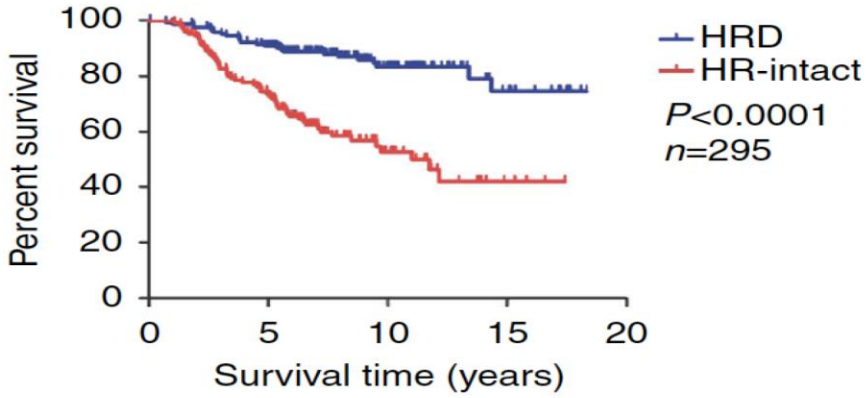
BRCA mutations

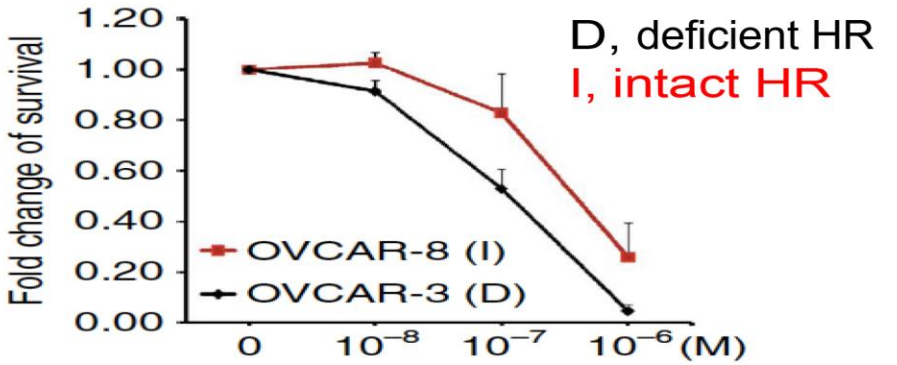
BRCA mutations… and beyond

Genes associated with mutations in Homolgous Recombination machinery



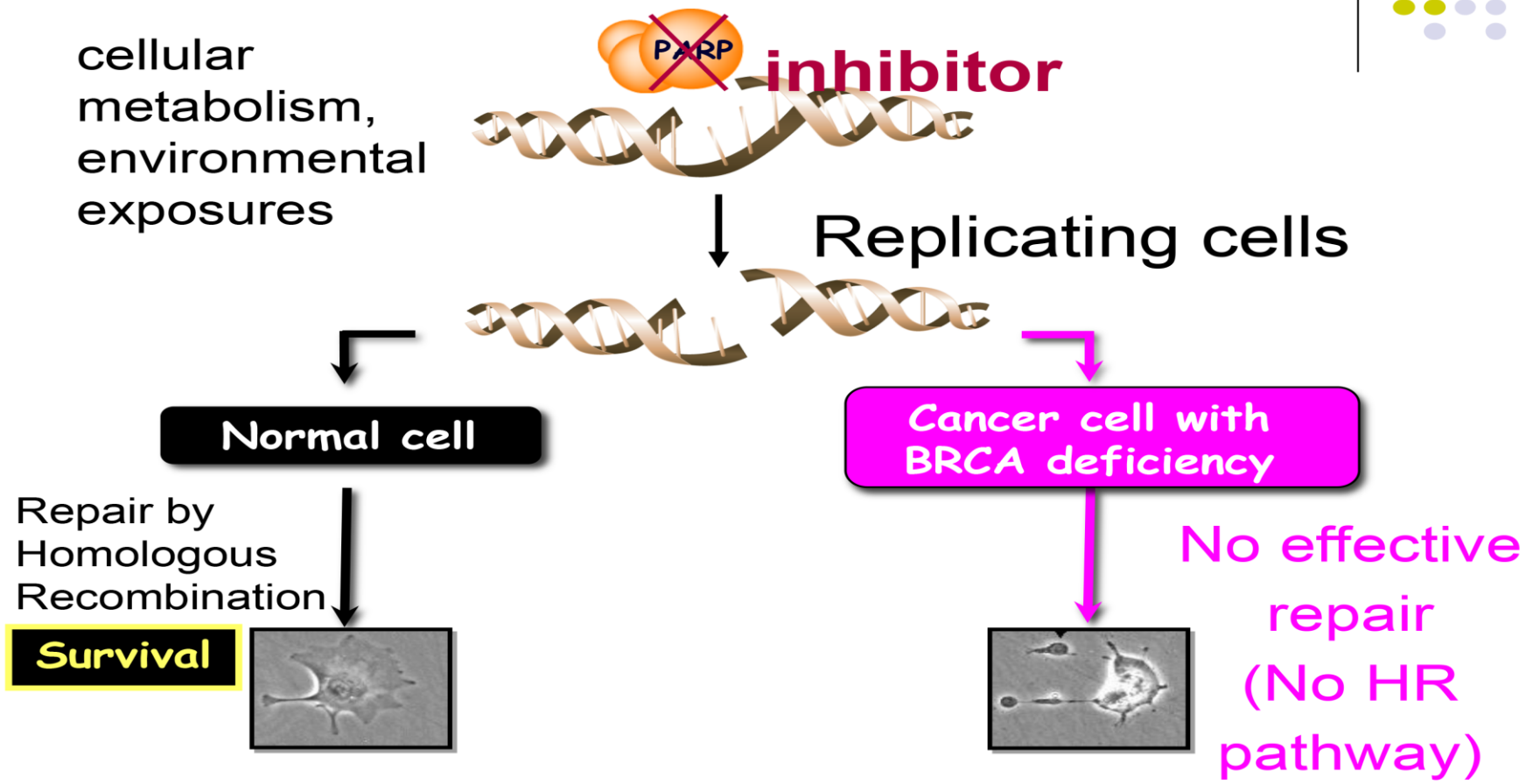
Peng et al, *Nat Comm*, 2014





PARP inhibition

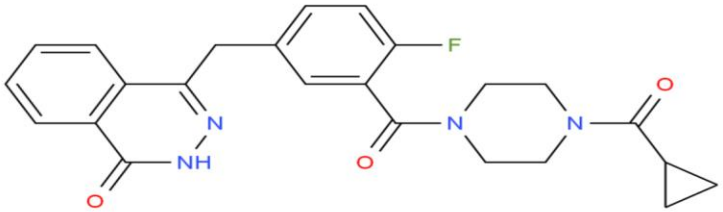
PARP inhibition: BRCA-mutant cancers

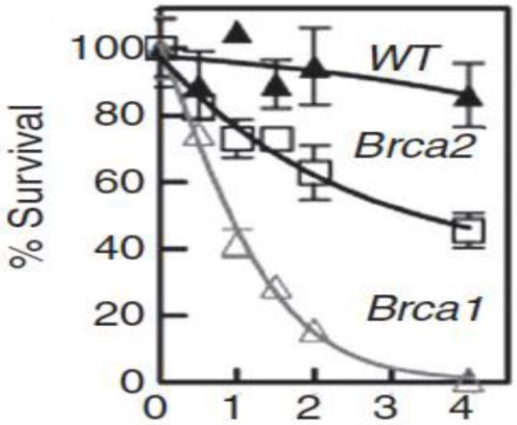


PARP inhibitor

PARP inhibitor

- Olaparib (AZD2281)
  - novel, orally active **PARP inhibitor**
  - synthetic lethality in homozygous BRCA-mut cells





Phase I/Ib study

Phase I/Ib Study of Olaparib and Carboplatin

Cohort 1
Br/Ov cancers
BRCA mutant
BRCApro ≥ 30%

(Lee, JNCI 2014)

- Olaparib 400mg twice daily (days 1-7)
- Carboplatin AUC 5 (every 21 days)

Cohort 2
TNBC
BRCA normal
BRCApro ≤ 10%

(Chiou, AACR 2014)

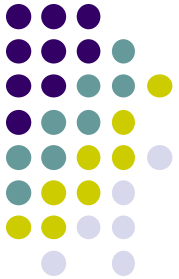
- Olaparib 400mg twice daily (days 1-7)
- Carboplatin AUC 4 (every 21 days)

Cohort 3
Serous Ovarian
BRCA normal
BRCApro ≤ 20%

(Chiou, ASCO 2015)

- Olaparib 400mg twice daily (days 1-7)
- Carboplatin AUC 4 (every 21 days)

Phase Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer



- **Results:** 45 enrolled patients
  - 37 ovarian cancer
  - 8 breast cancer

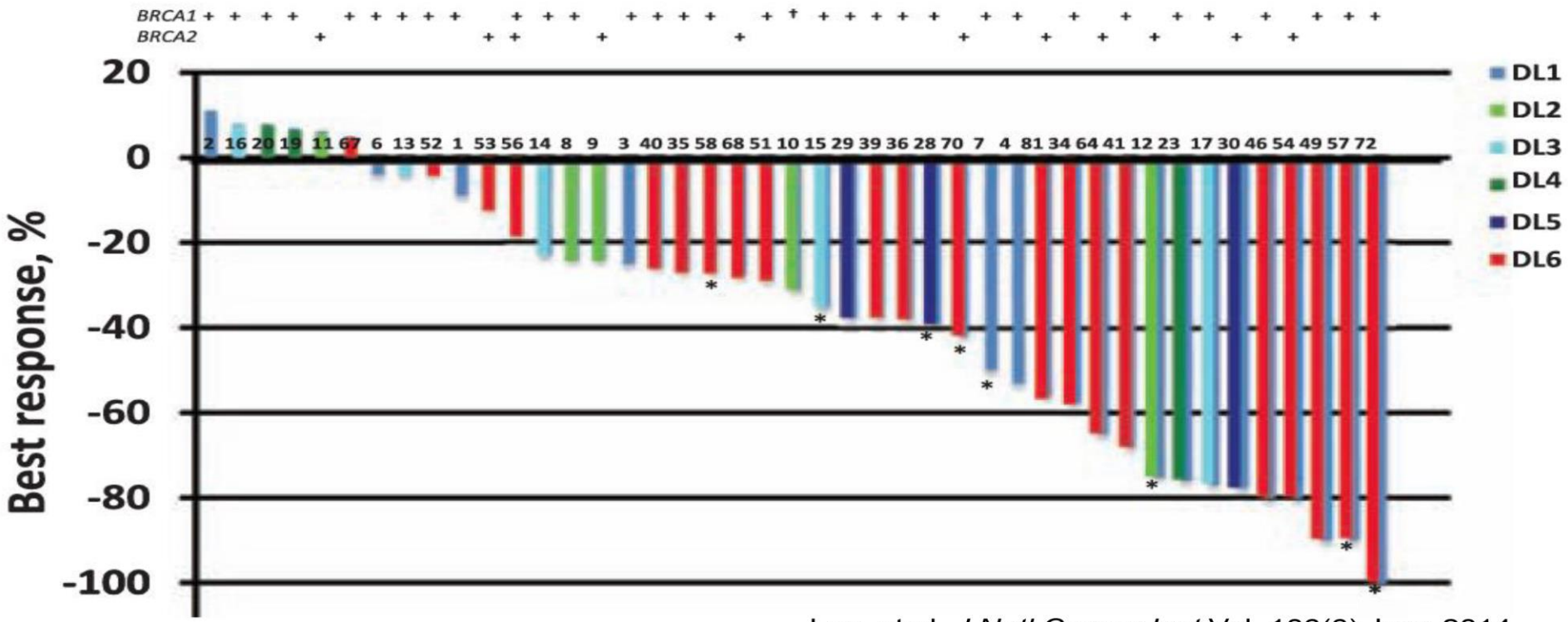
  - Phase 1 dose escalation = 30 patients
  - Phase 1b expansion = 15 patients

  - **MTD =** Carboplatin AUC5 on day 1 + Olaparib 400mg twice daily on days 1-7, every 21 days

Lee, et al. *J Natl Cancer Inst* Vol. 106(6) June 2014

Phase 1b Study

Phase Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer



Lee, et al. *J Natl Cancer Inst* Vol. 106(6) June 2014

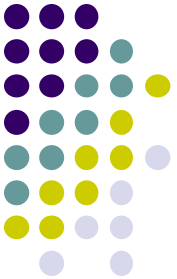
Phase 1b study

Phase Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer

	Ovarian cancer (n = 34)†	
Best response	**No. (%)**	**Median duration in months (range)**
CR	0	
PR	15 (44.1)	16 (4 to >45)
SD ≥ 4 mo	13 (38.2)	11 (6 to 24)
PD	6 (17.6)	
Overall response rate	15/34 (44.1)	
Clinical benefit rate	28/34 (82.3)	

Lee, et al. *J Natl Cancer Inst* Vol. 106(6) June 2014

Phase Ib Study of Olaparib and Carboplatin in BRCA1 or BRCA2 Mutation-Associated Breast or Ovarian Cancer

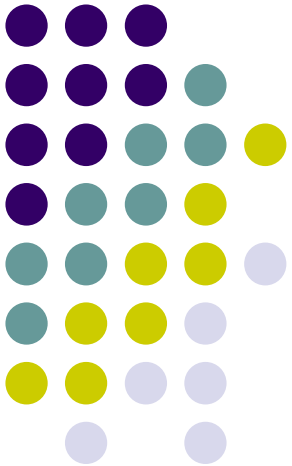


- **Conclusions:**
  - Oral olaparib is well tolerated in combination with carboplatin
  - Highly active in advanced, chemotherapy-refractory BRCA-deficient cancer
  - Greater activity seen at the higher dose
  - Positive proof of the concept of the activity and tolerability of **genetically defined targeted therapy** with olaparib in BRCA-deficient cancers
  - Results of sporadic HGSOC cohort to be presented at ASCO meeting 2015

Lee, et al. *J Natl Cancer Inst* Vol. 106(6) June 2014

Exploration of new targets

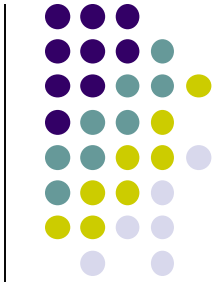
Functional Genomics



“Actionable” mutations

- Commercially available testing
  - e.g., Caris, Foundation One
  - Report “possible” or “unlikely” benefit
- “Basket” clinical trials
  - e.g., NCI-MPACT
  - Assign treatment based on mutation
- Typically no functional link

“Actionable” mutations



- “…depends in large part on the strength of the data linking the target and targeted therapy.”
- “For this trial design to work, two key conditions must be met:
  - the tumor must depend on the target pathway, and
  - the targeted therapy must reliably inhibit the target.”
- “Achieving both goals can be a matter of some complexity.”

“Actionable” targets

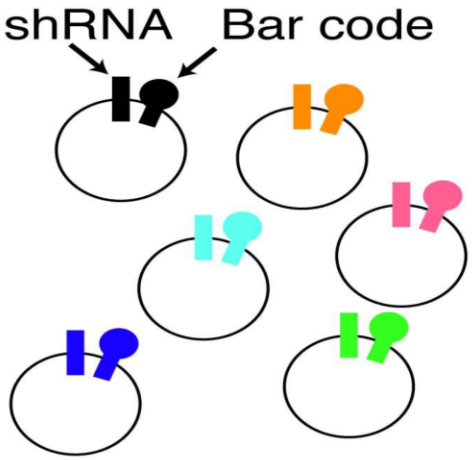
- Need a functional experiment

- Functional genomics

Functional genomics

Using a functional genomics screen to identify targets

Creation of an Inducible shRNA Retroviral Library for Functional Genomics Studies of Cancer Phenotypes

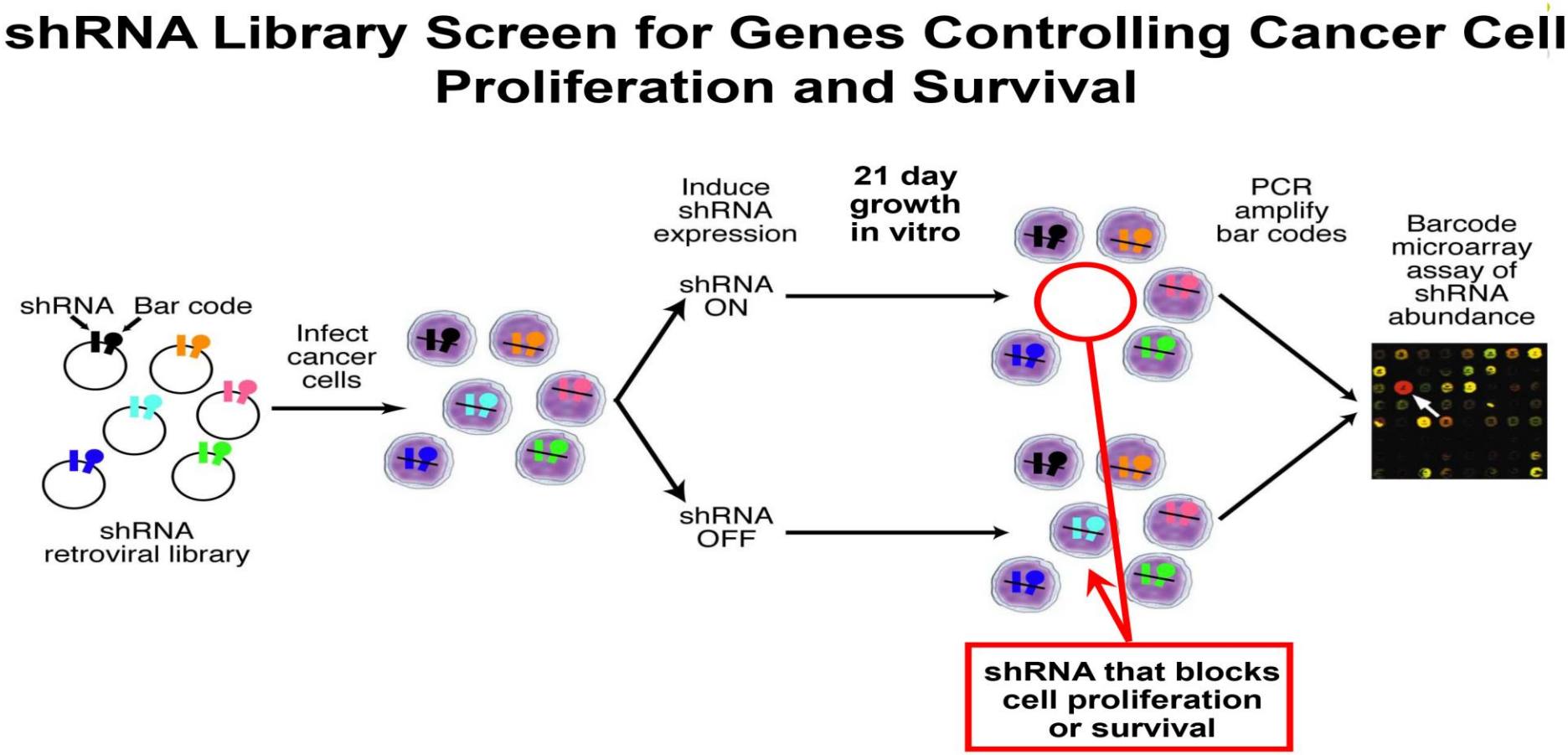


shRNA retroviral library

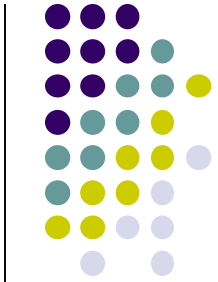
-- shRNAs targeting **2500** human genes
-- 3 shRNA constructs per gene
-- All sequence verified
-- All containing identified 60-mer bar code sequence
-- shRNA expression is inducible by doxycycline
-- Library target genes:
- All protein kinases
- All PI3 kinase
- All deubiquitinating enzymes
- NF-kB pathway regulators
- Differentially expressed genes among lymphoma types
- Apoptosis regulators, oncogenes, tumor suppressors

Ngo, et al. *Nature* 2006

shRNA Library Screen

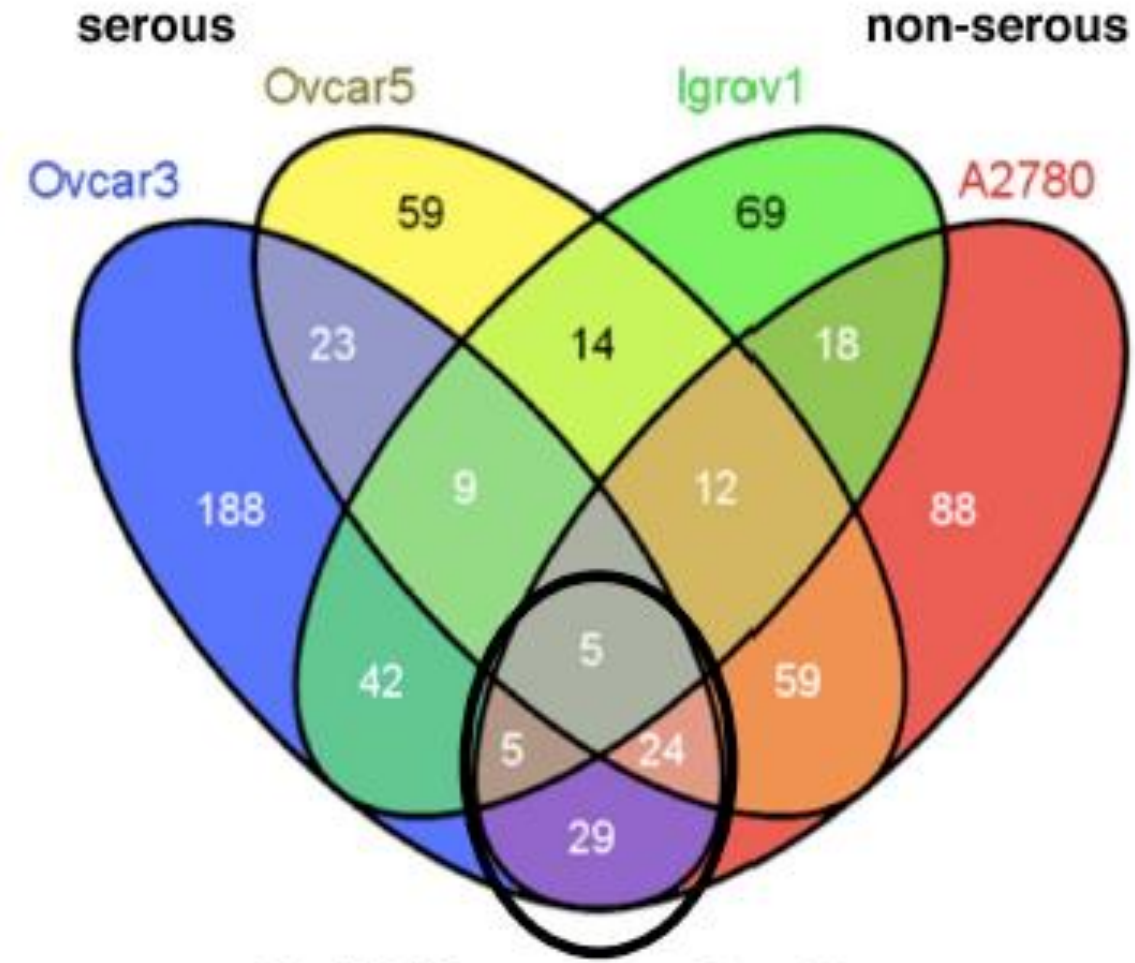


Functional Genomics of ovarian cancer



- Four ovarian cancer cell lines
  - OVCAR3 – serous
  - OVCAR5 – serous
  - Igrov1 – non-serous
  - A2780 – non-serous

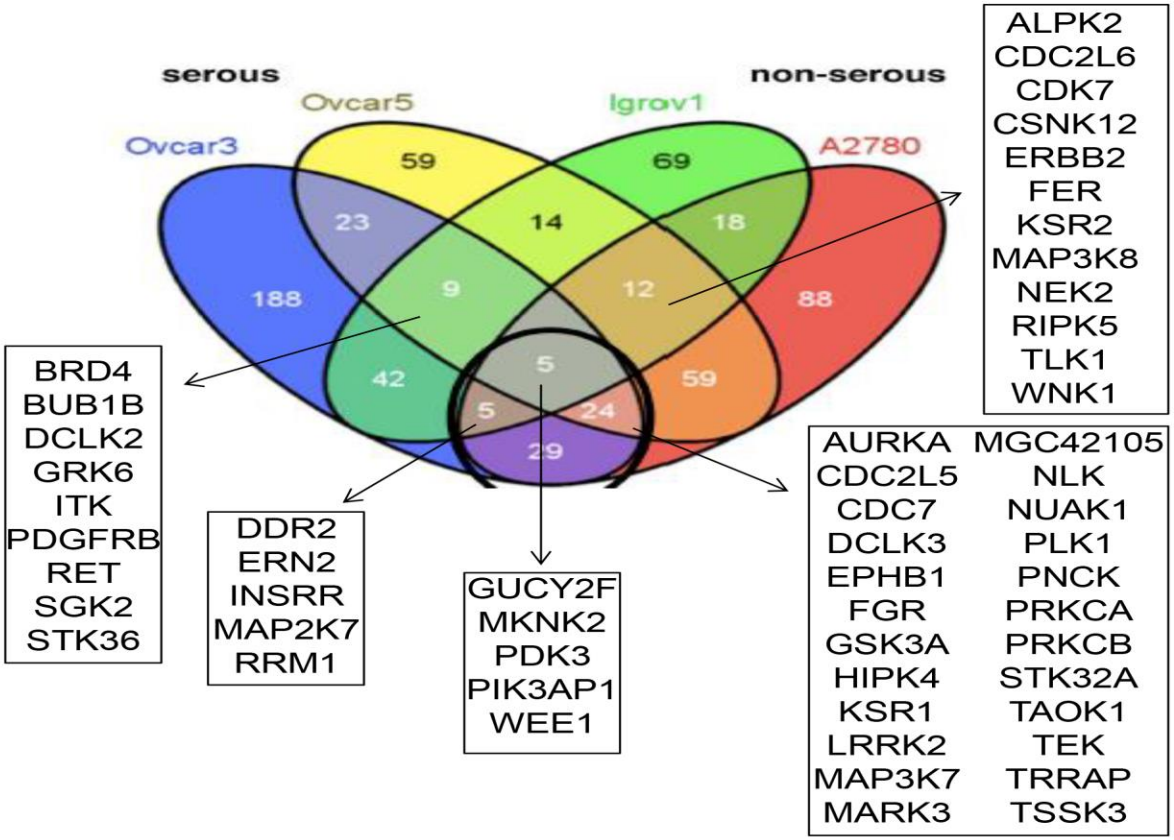
Common targets in ovarian cancer – “drivers”?



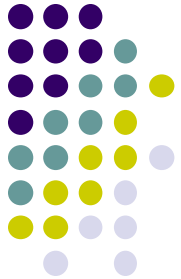
63 shRNAs representing 55 genes

Common targets

Common targets in ovarian cancer – “drivers”?



Functional genomics of ovarian cancer



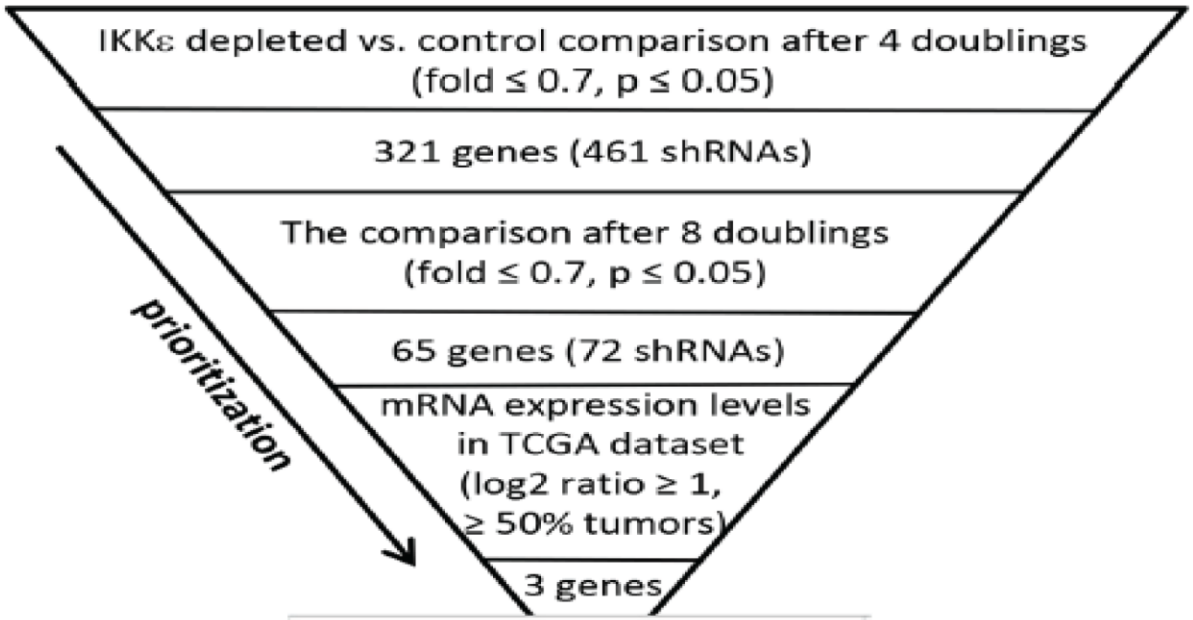
- Following up in
  - 6 additional cell lines
  - 2 different RNAi constructs
  - Select “druggable” targets

- Focused functional screens
  - Specific subgroup of serous ovarian cancer
  - NF-kappaB signaling pathway

CHEK1

CHEK1

- Highly synergistic with IKKε
- Over-expressed in nearly all ovarian cancers

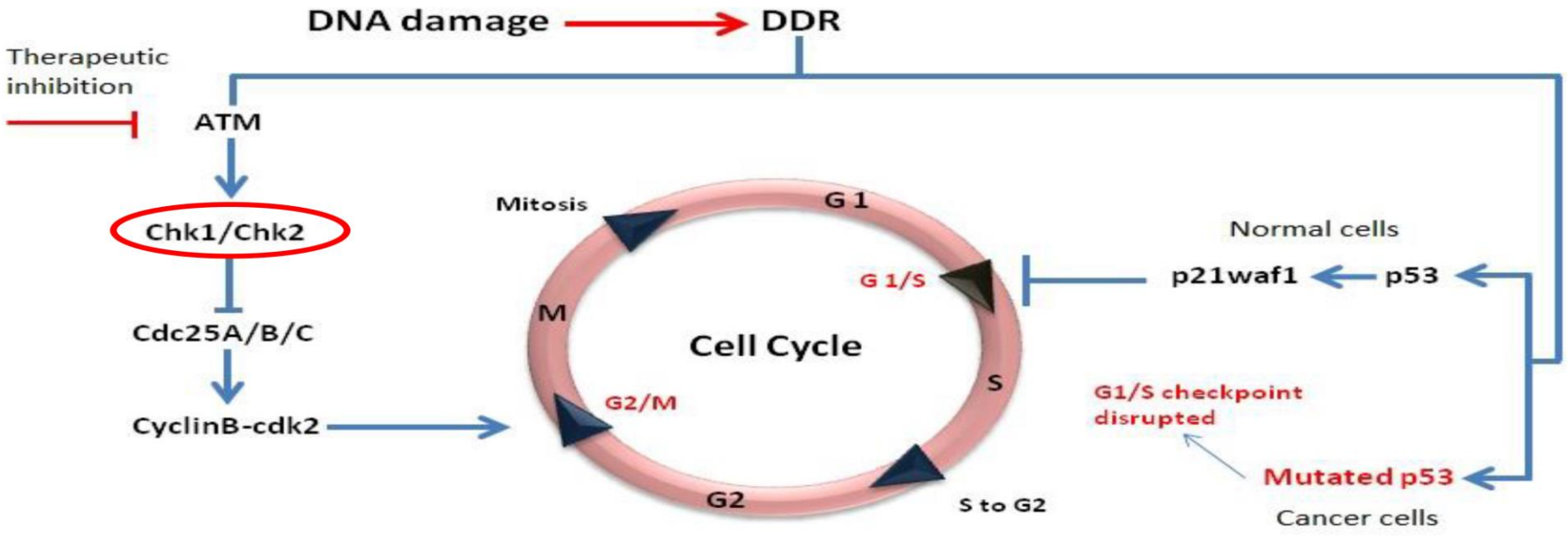


TCGA Expression	
Gene	Log2 T/N ratio≥1
CHEK1	496/506 (98%)
EPHB3	413/506 (82%)
PIP5K1A	265/506 (52%)

Kim, et al. *Oncotarget*, 2014

CHEK signaling

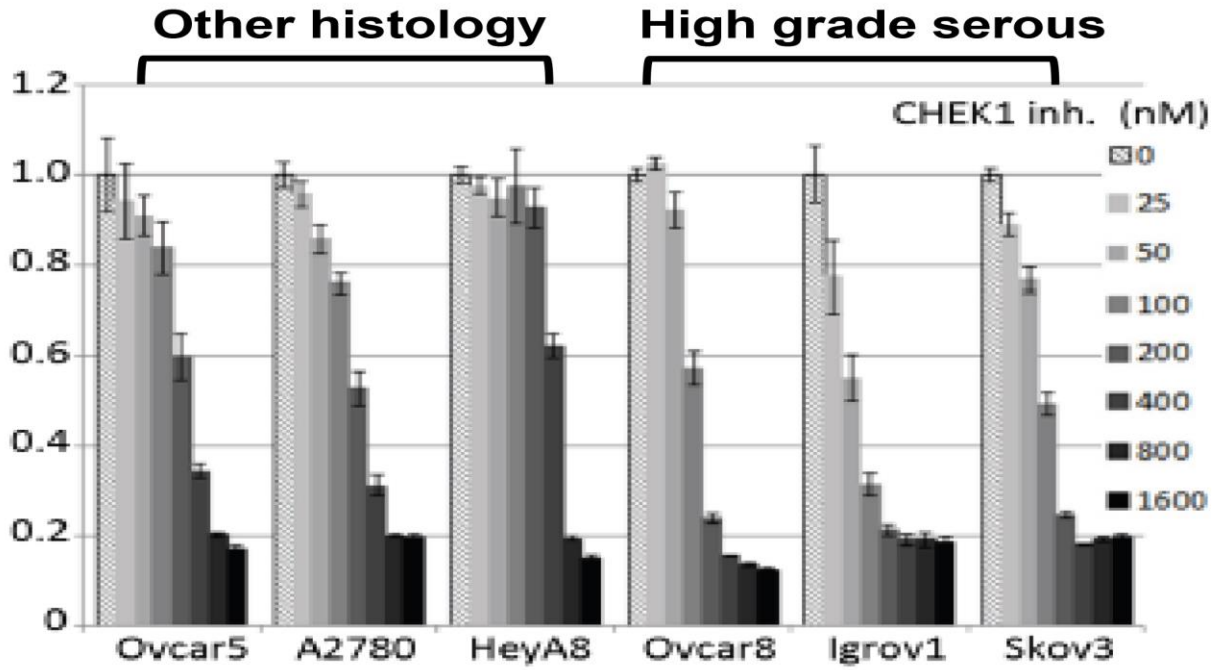
CHEK signaling



CHEK inhibitor

CHEK inhibitor

- Most potent in HGSOC



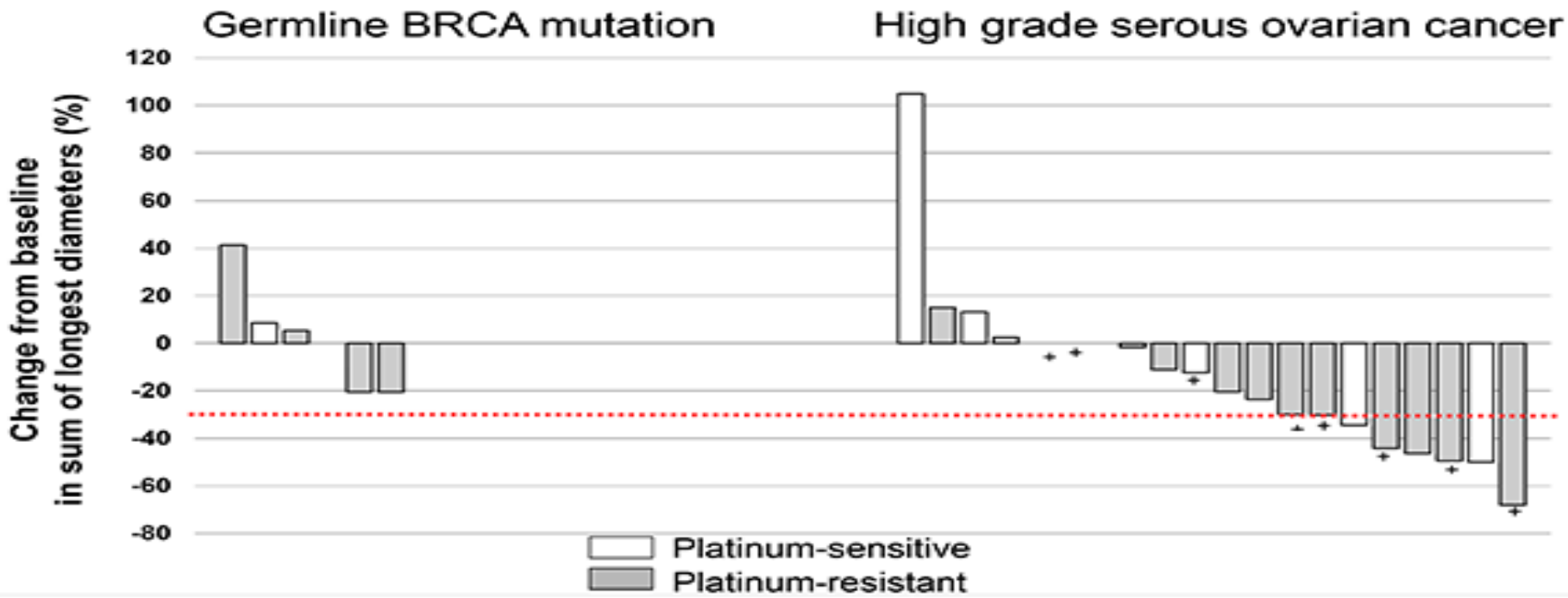
Kim, et al. *Oncotarget*, 2014

CHEK inhibitor

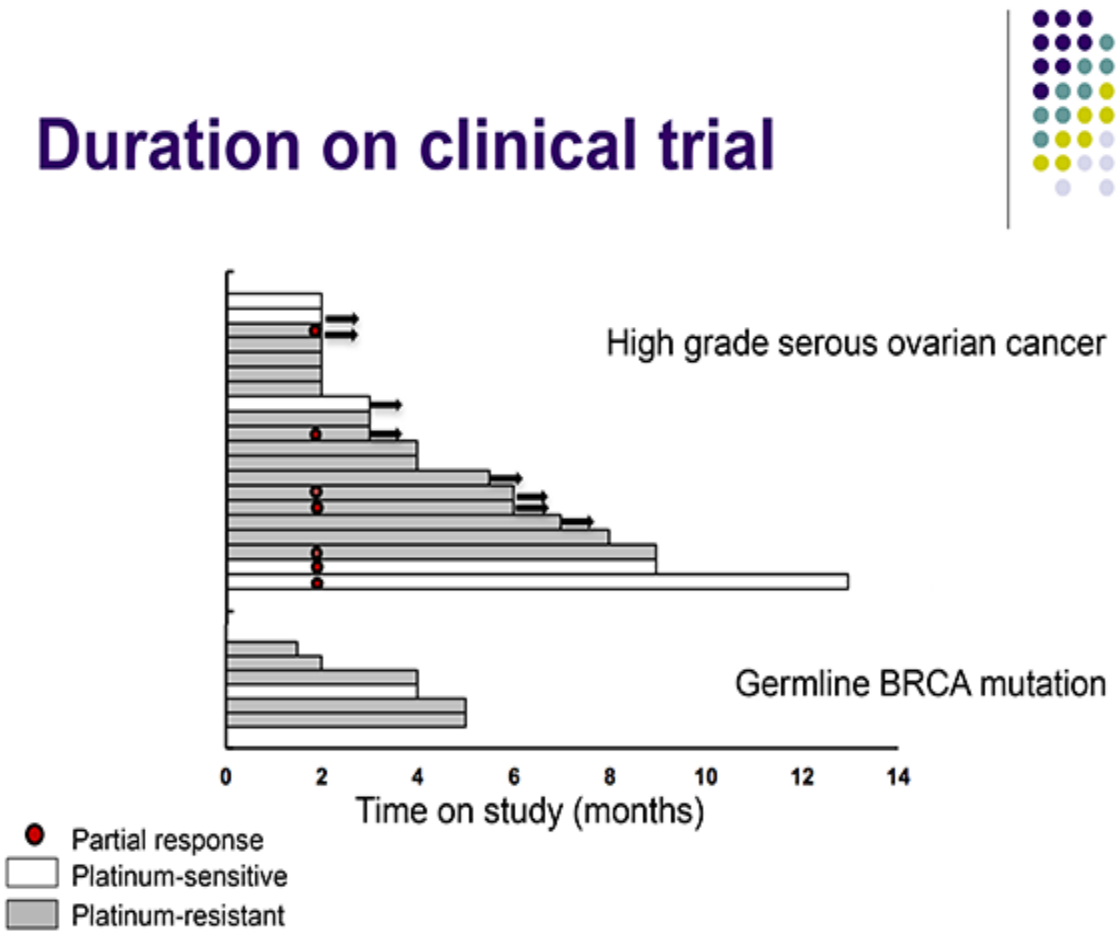
- Clinical trial ongoing
  - NCT02203513
  - Promising results in High grade serous non BRCA
  - Highlighted by a Functional Genomics approach

Best response on clinical trial

Best response on clinical trial

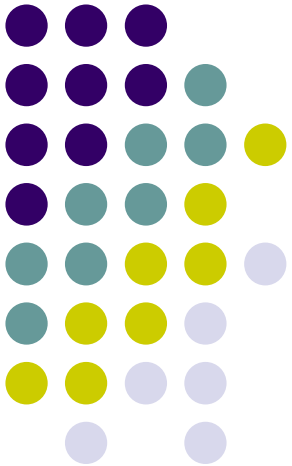


Duration on clinical trial



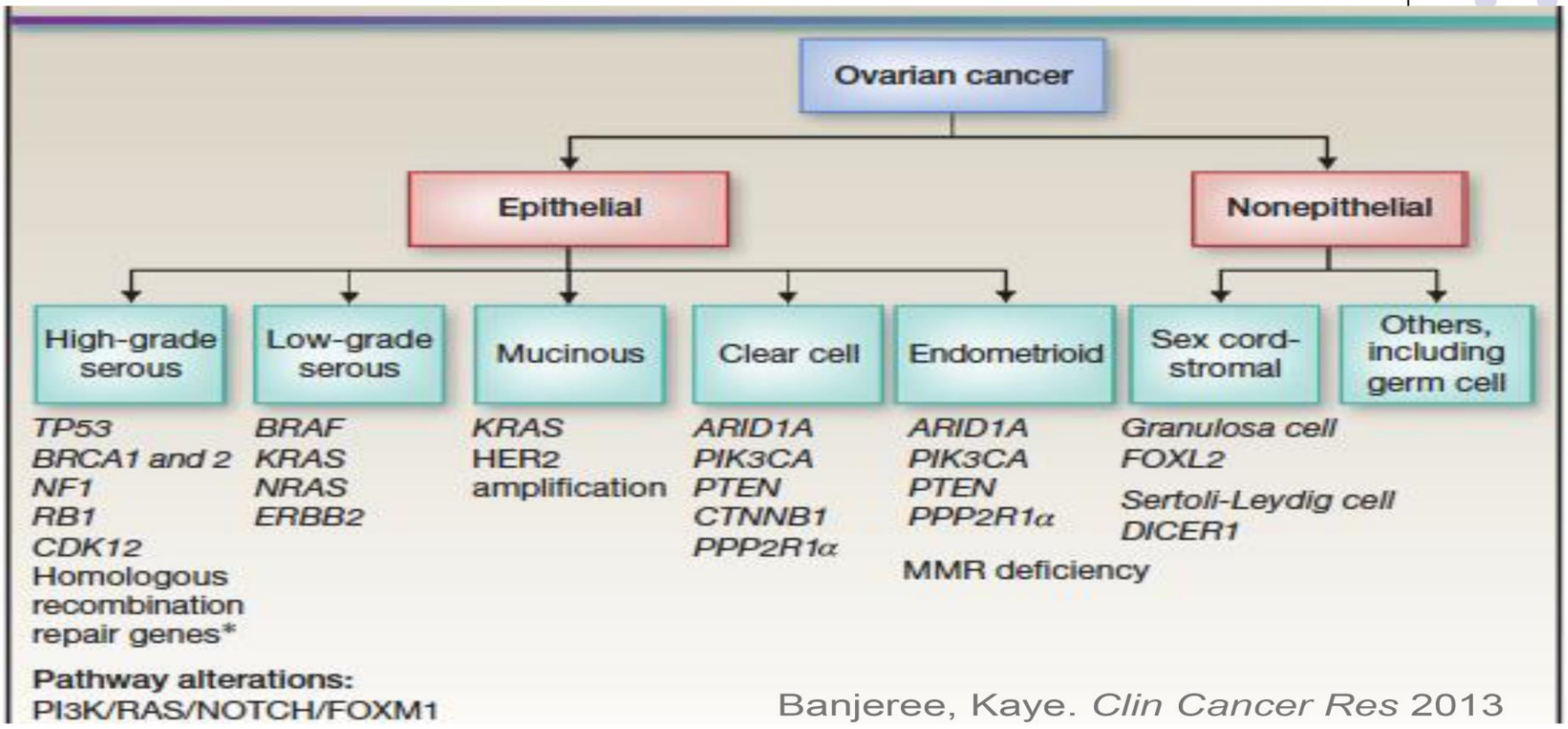
Ovarian cancer genomics

Summary



Ovarian cancer genomics

Ovarian cancer genomics



Functional Genomics

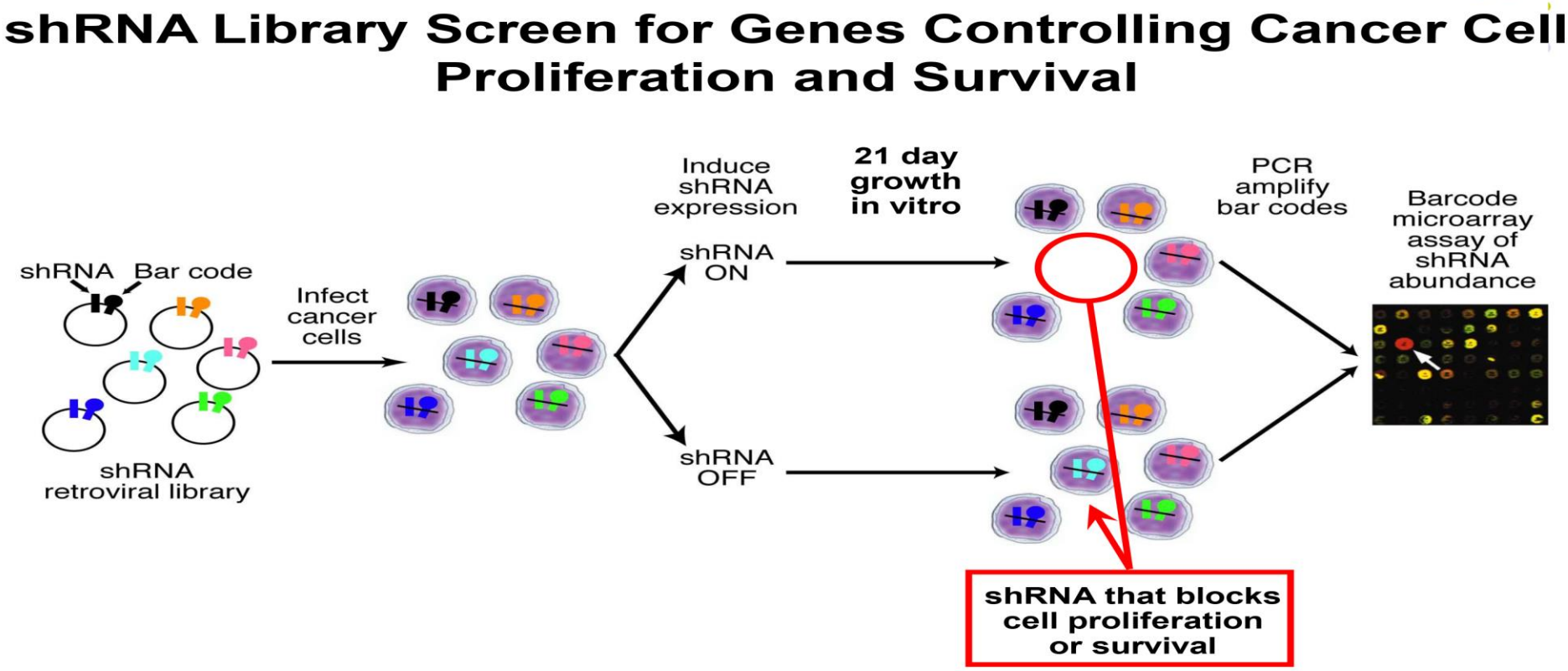
- 1981 – Shih – discovery of Her2/neu

Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts

Chiaho Shih, L. C. Padhy, Mark Murray & Robert A. Weinberg

Department of Biology and Center for Cancer Research

Controlling genes



Ovarian cancer and genomics

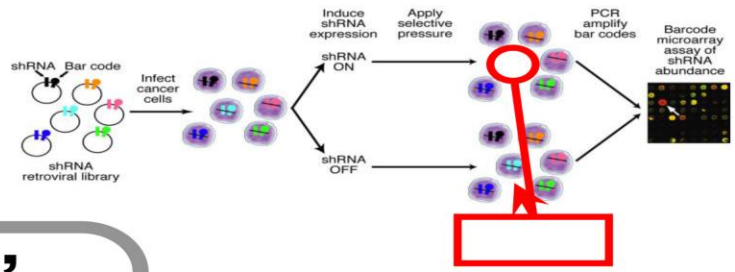
Ovarian Cancer in the Genomics Era

Functional genomic screen

“Driver” aberration/pathway

Clinical trial

shRNA Library Screen for Genes Controlling Cancer Phenotypes



Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts

Chiaho Shih, L. C. Padhy, Mark Murray & Robert A. Weinberg

Women's cancer team