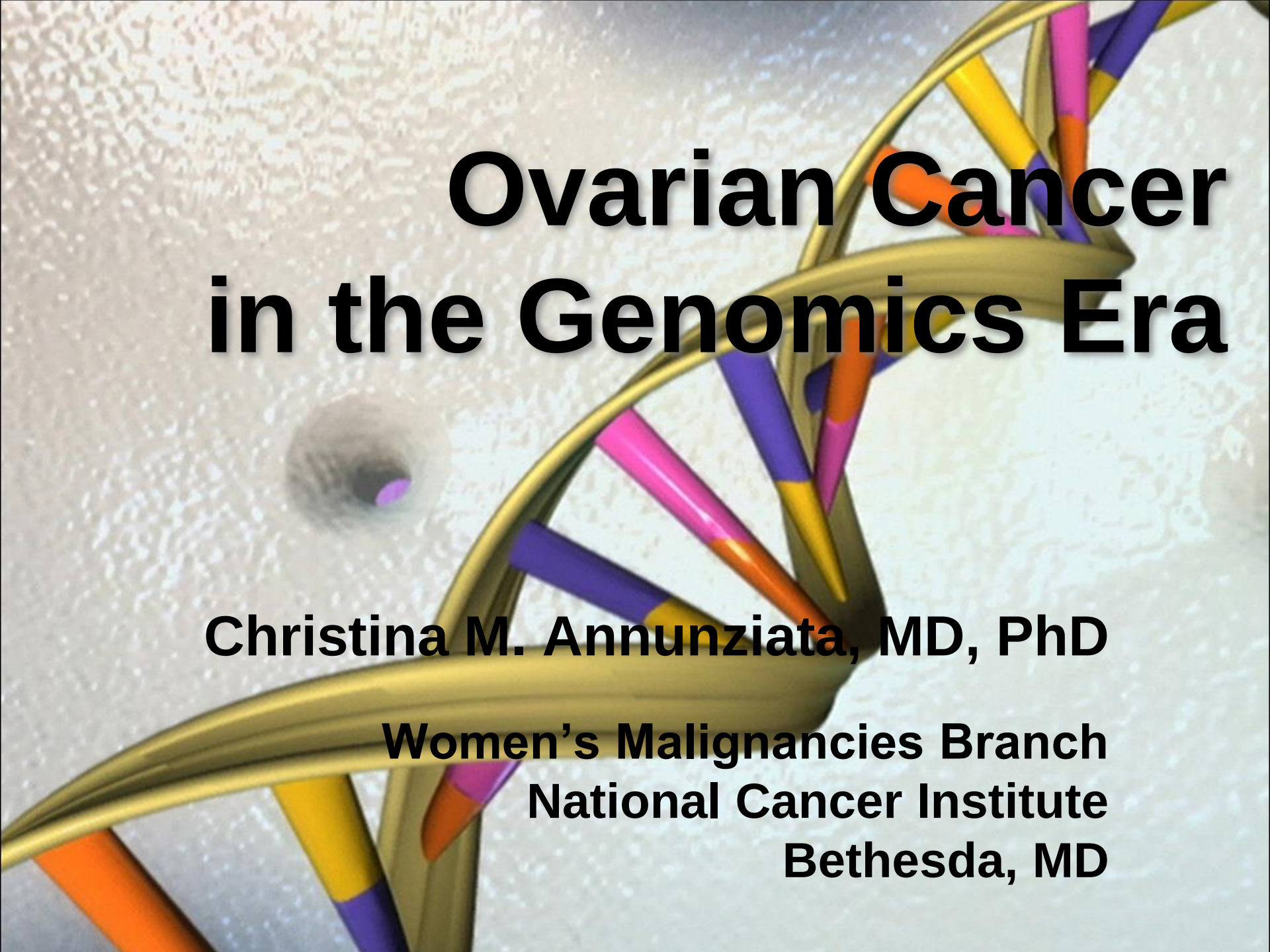
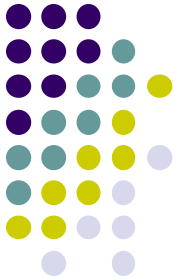


Ovarian Cancer in the Genomics Era

Christina M. Annunziata, MD, PhD

Women's Malignancies Branch
National Cancer Institute
Bethesda, MD

Cancer Genomics



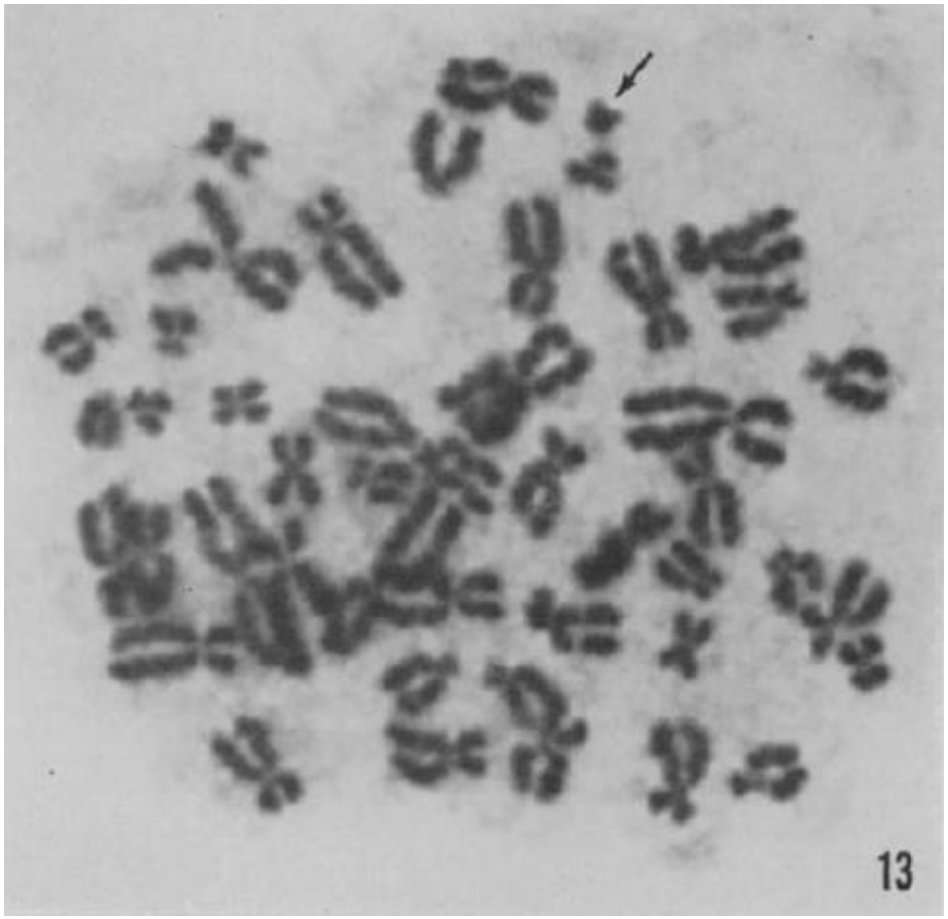
- Study of the genome
  - Chromosomes
  - Gene expression
  - Global analysis (not individual entities)

The Genomics Era

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

The Genomics Era

- 1959 – Nowell and Hungerford



The Genomics Era

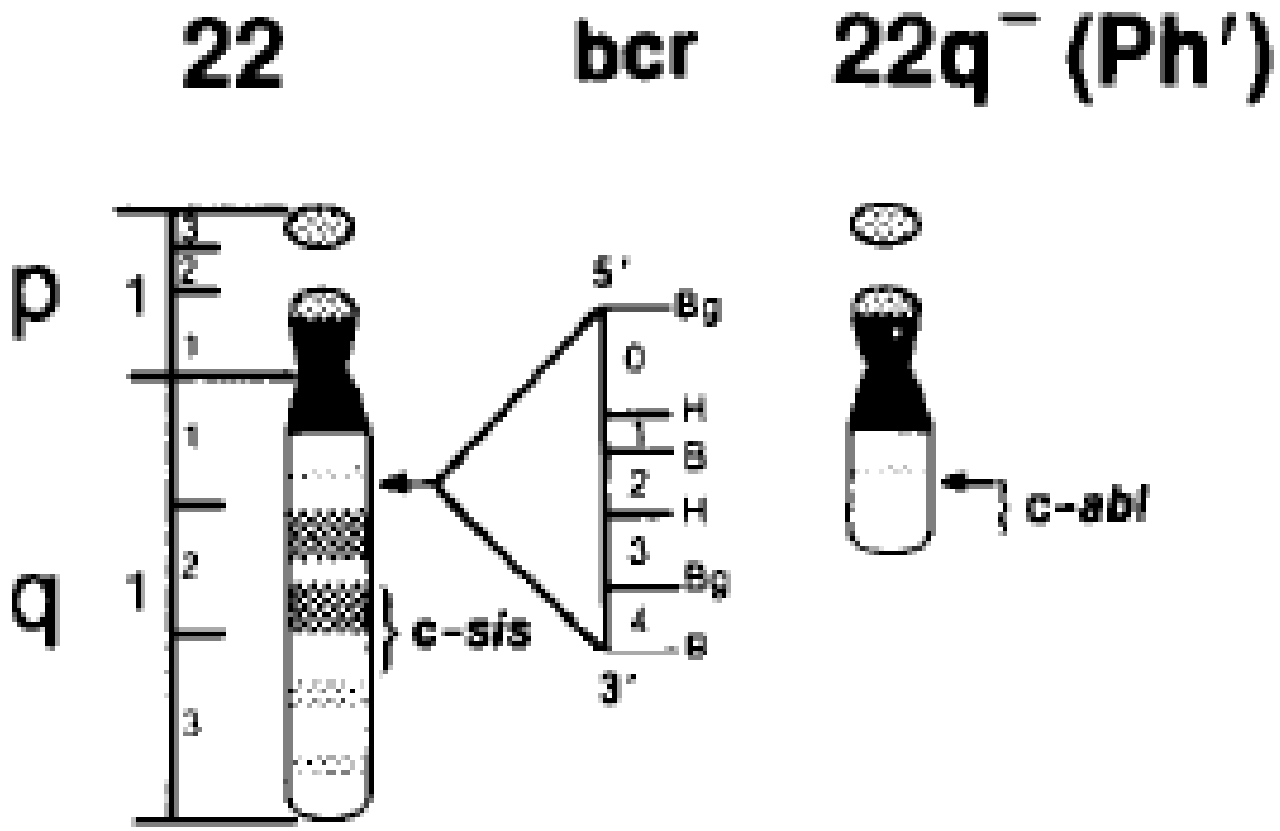
- 1973 – Janet Rowley

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	1⅓	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

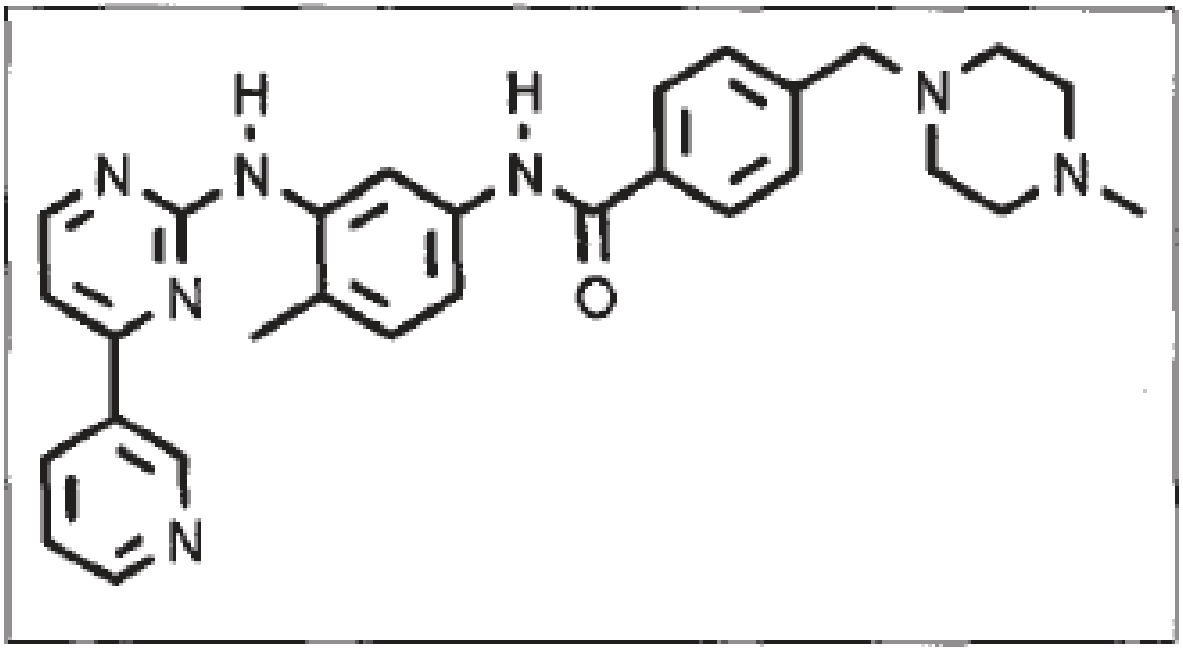
- 1984 – Groffen – BCR-ABL



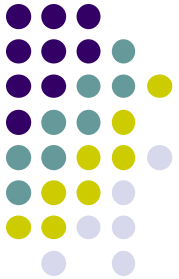
The Genomics Era

- 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

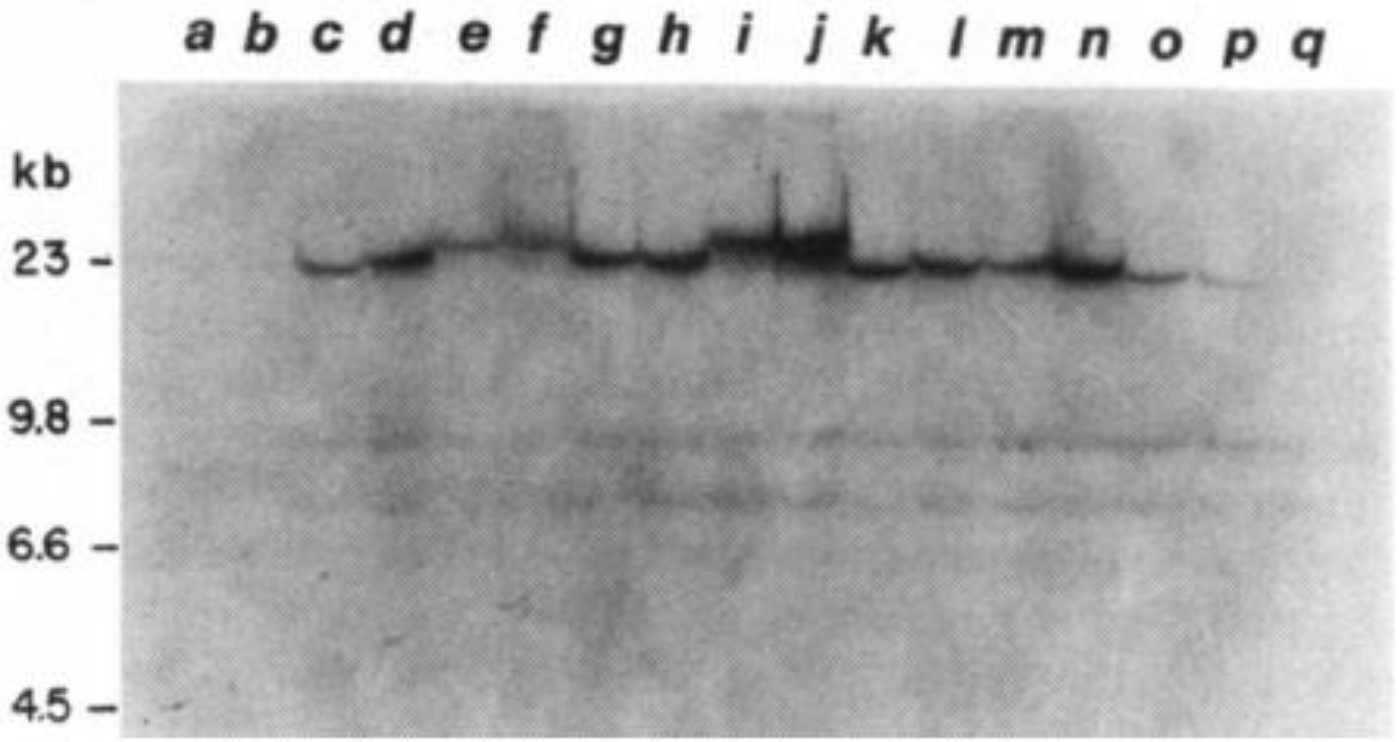
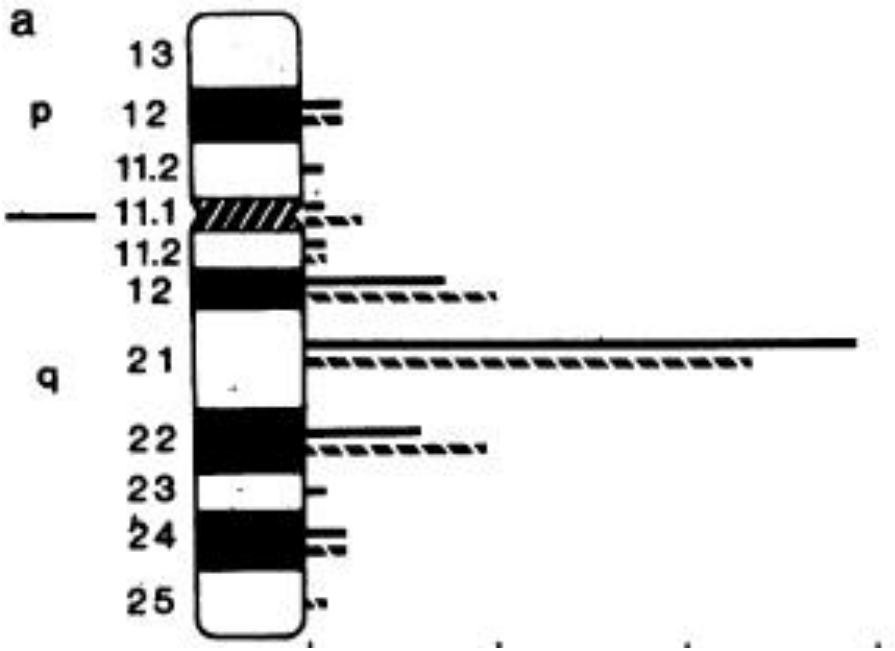
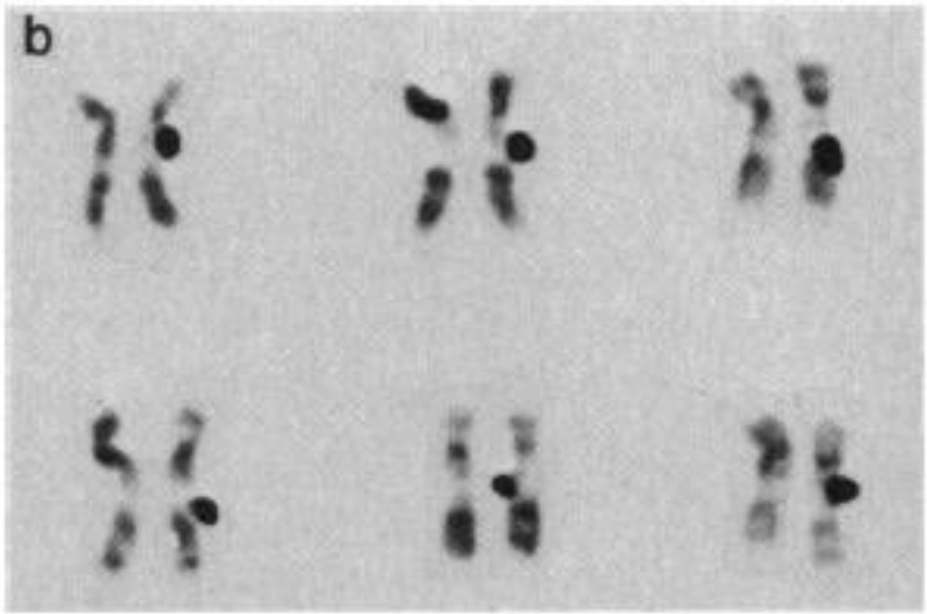


Fig. 1 Southern blot analysis of *erb-B*-related sequences in NIH 3T3 cells transformed with rat neuro/glioblastoma DNAs;

Functional Genomics

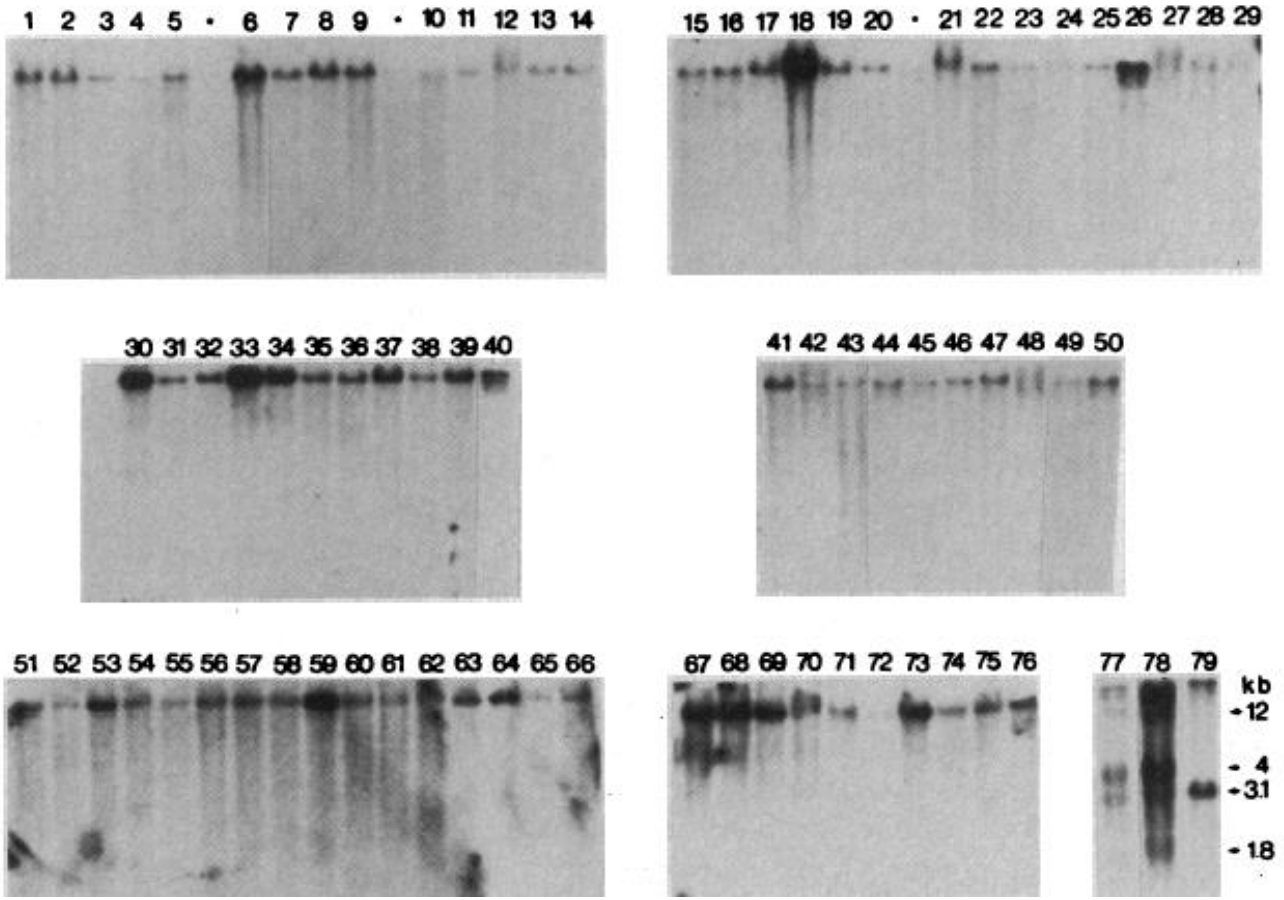
- 1985 – Coussens – Her2 on chromosome 17





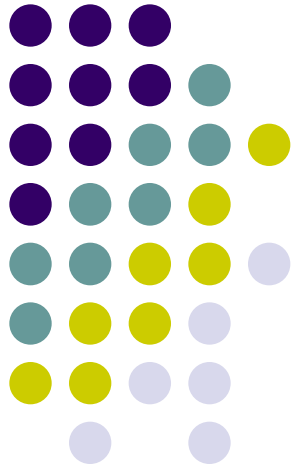
Functional Genomics

- 1987 – Slamon – HER2 in breast cancer

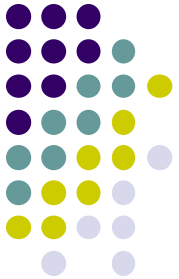


Using genomics to study ovarian cancer

Do we have any “drivers”?

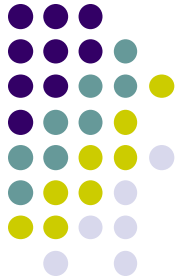


Ovarian Cancer



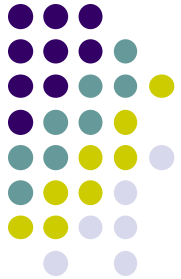
- Most lethal gynecologic malignancy in the US
  - >16,000 deaths/yr
  - 5th most common cancer death for women
- 70% diagnosed with advanced disease
- < 35% of advanced stage patients alive at 5y

Ovarian Cancer



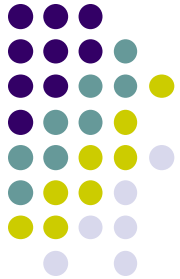
Stage	Description	Incidence	Survival
I	Confined to ovaries	20%	90%
II	Confined to pelvis	5%	65%
III	Spread IP or nodes	58%	45%
IV	Distant metastases	17%	<5%

Treatment for Newly Diagnosed Ovarian Cancer



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

The State of Treatment for Newly Diagnosed Ovarian Cancer

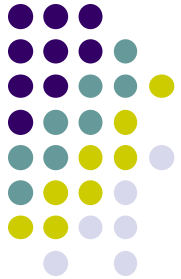


- Complete surgical staging
- Optimal reductive surgery
- **Chemotherapy**
  - Platinum = cisplatin or carboplatin

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

- *Clinical Trials*

Treatment and Outcome for Advanced Ovarian Cancer



ALKYLATORS

CISPLATIN/ALKYLATOR COMBINATIONS

INTRA-PERITONEAL

1960 1980 2000

1970 1990

CISPLATIN

PACLITAXEL/ CARBOPLATIN

0 5% 15% 35% 40%

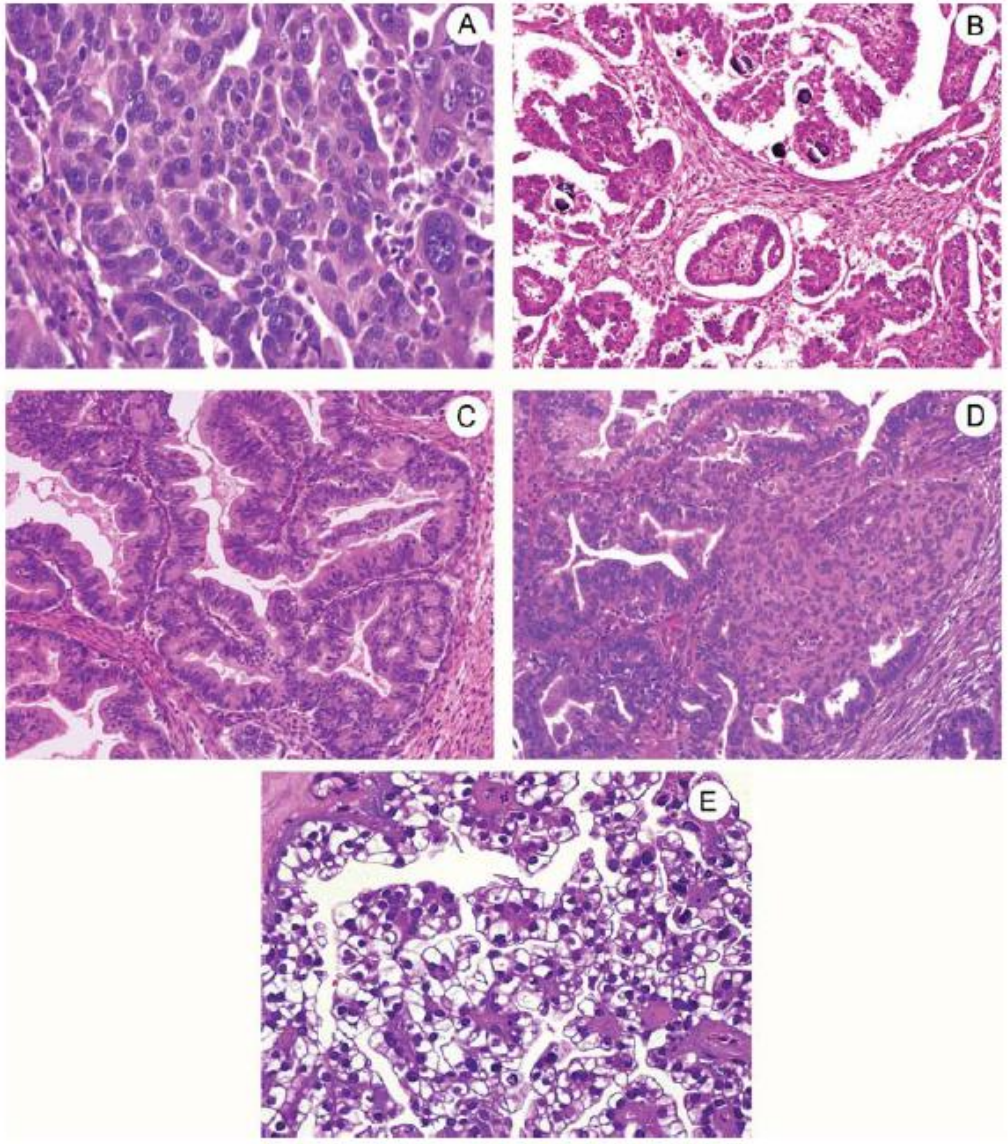
1960 1970 1980 1990 2000

5 YR SURVIVAL ADVANCED DISEASE

Ovarian Cancer

Prevalence

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



Soslow R. *Int J Gyneol Pathol*, 2008

Ovarian Cancer

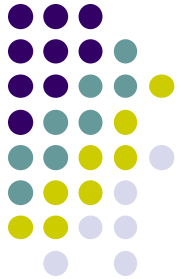
Prevalence

- 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

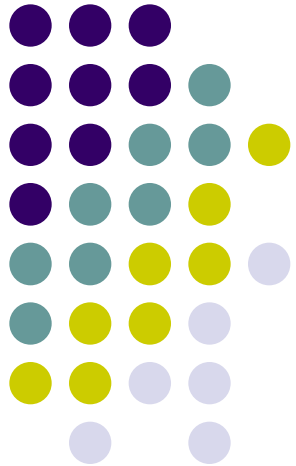
Ovarian Cancer



- 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

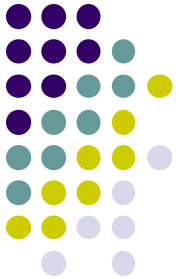
- 5-10% of all cases (serous = 70%)
- Worse response to standard chemotherapy
- Associated with endometriosis (up to 40%)

Clear cell 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

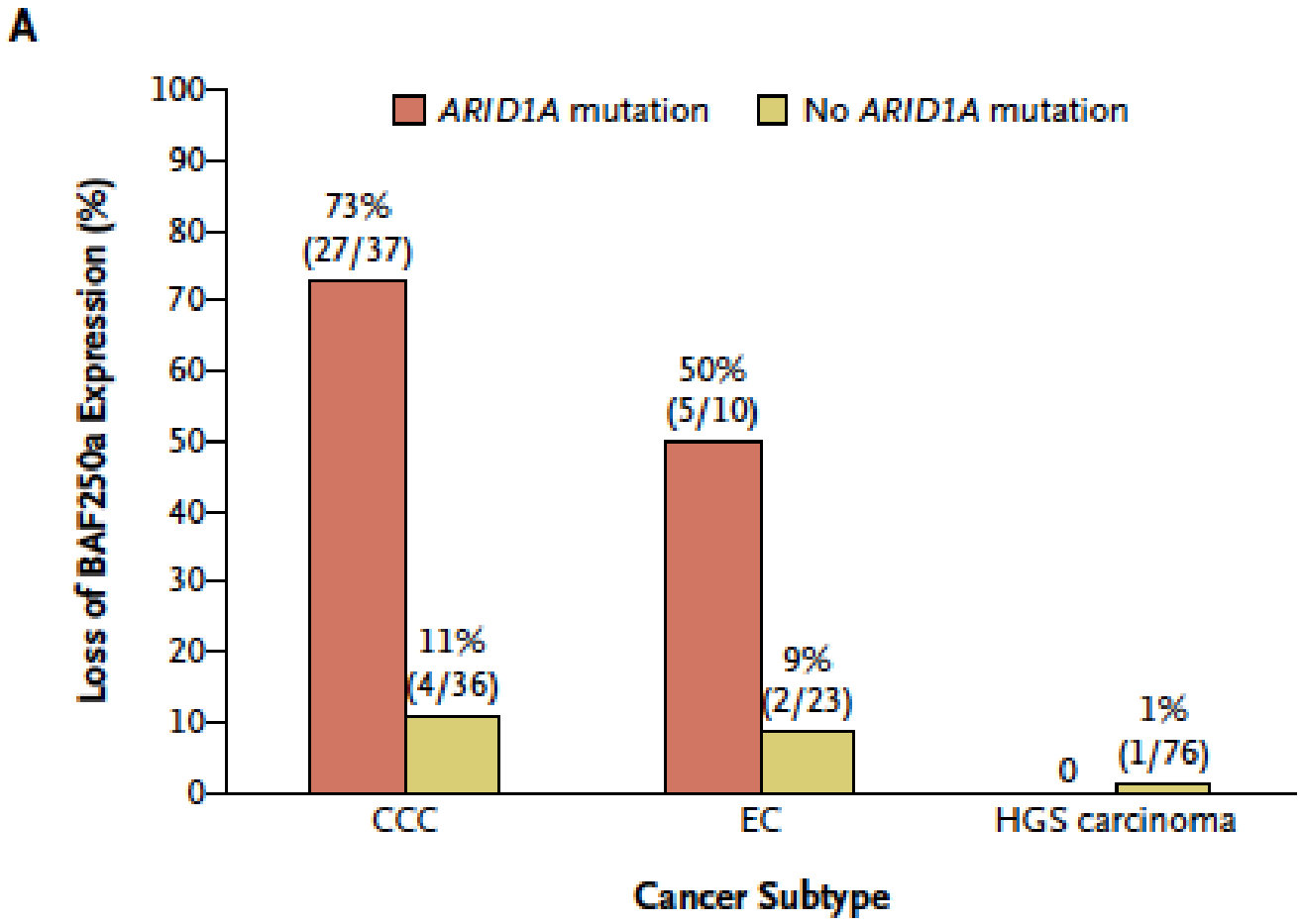


Weigand, *NEJM* 2010

ARID1A

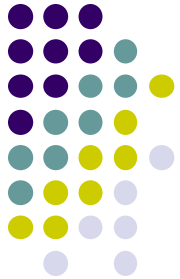
- SWI-SNF chromatin remodeling complex
- Mutated in breast cancer, lung cancer
- 1p36: deleted 6% of all cancers
- Tumor suppressor gene?

ARID1A mutations



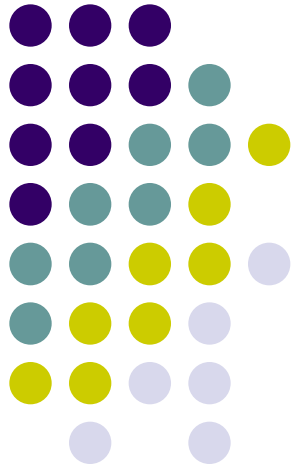
Weigand, *NEJM* 2010

Clear cell and endometrioid cancer

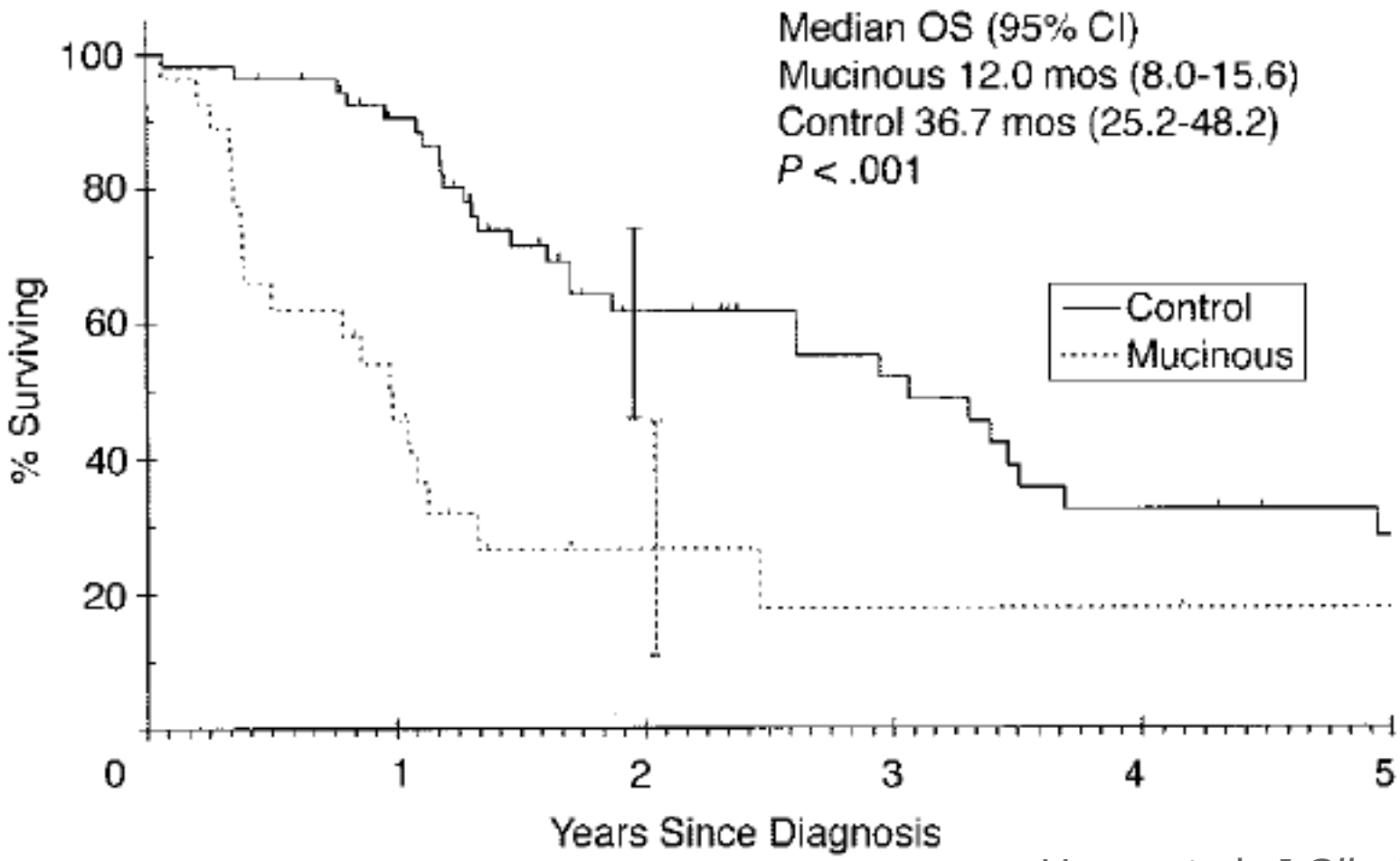


- ARID1A mutated or lost in
  - Over 40% clear cell
  - 30% endometrioid
  - Less than 1% serous
- Unknown oncogenic mechanism
  - No indication of which resulting pathways affected
  - Unclear therapeutic utility
- Diagnostic utility?
  - Not a ‘functional’ experiment

Mucinous

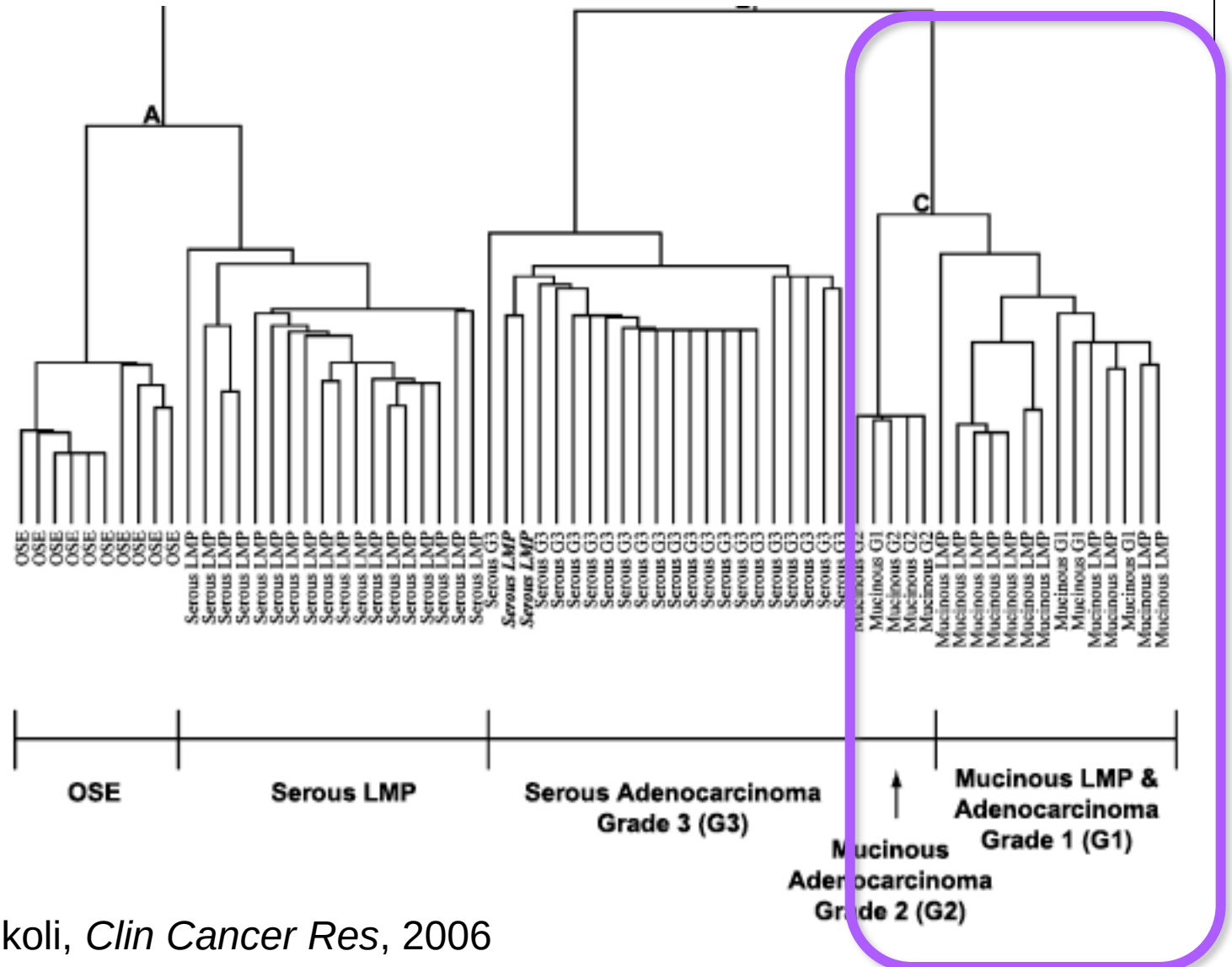


Mucinous ovarian cancer



Hess, et al. *J Clin Oncol*, 2004

Gene expression – mucinous versus serous



Wamunyokoli, *Clin Cancer Res*, 2006

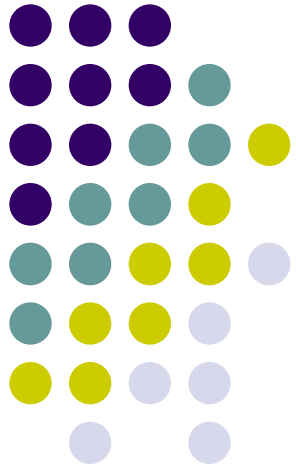
KRAS mutations - mucinous

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



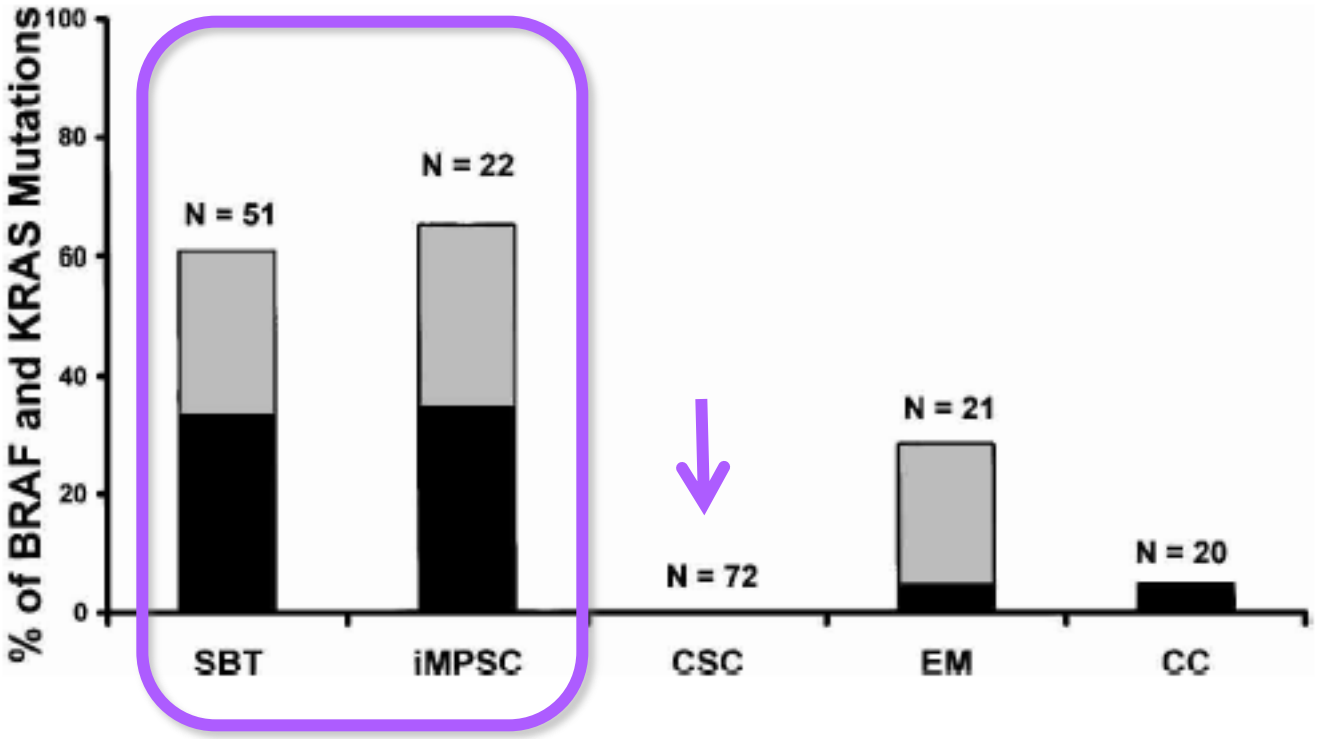
KRAS and BRAF mutations

- 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 mutations



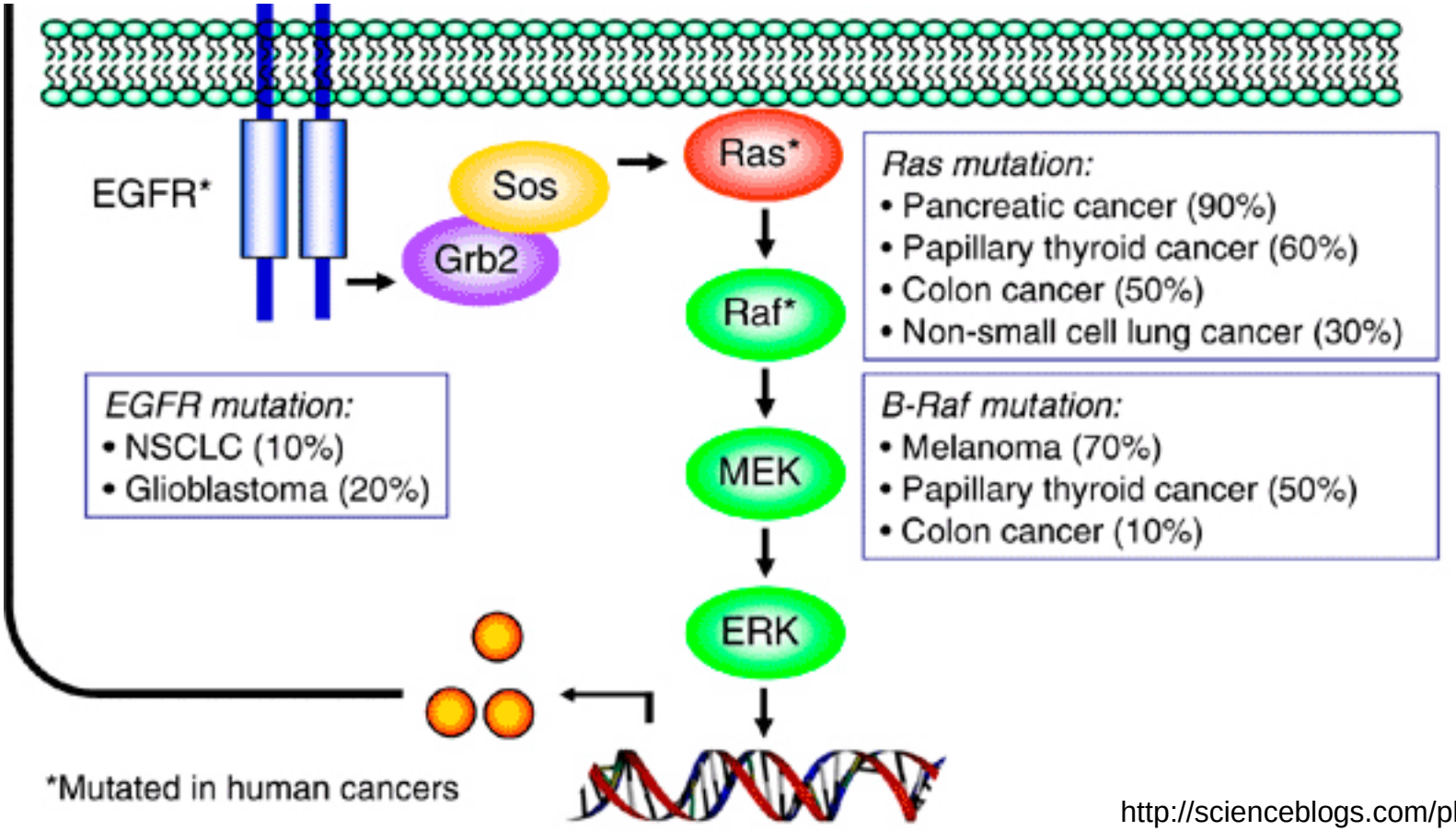
Serous borderline tumors

Invasive low grade serous cancers

High grade serous cancers

Singer, *JNCI* 2003

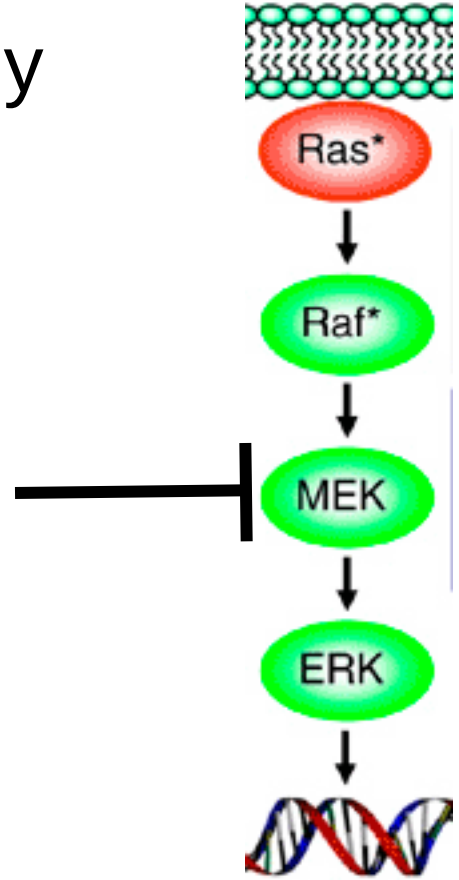
RAS signaling pathway
- a potential driver?



http://scienceblogs.com/pharyngula/2013/09/21/16271/

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

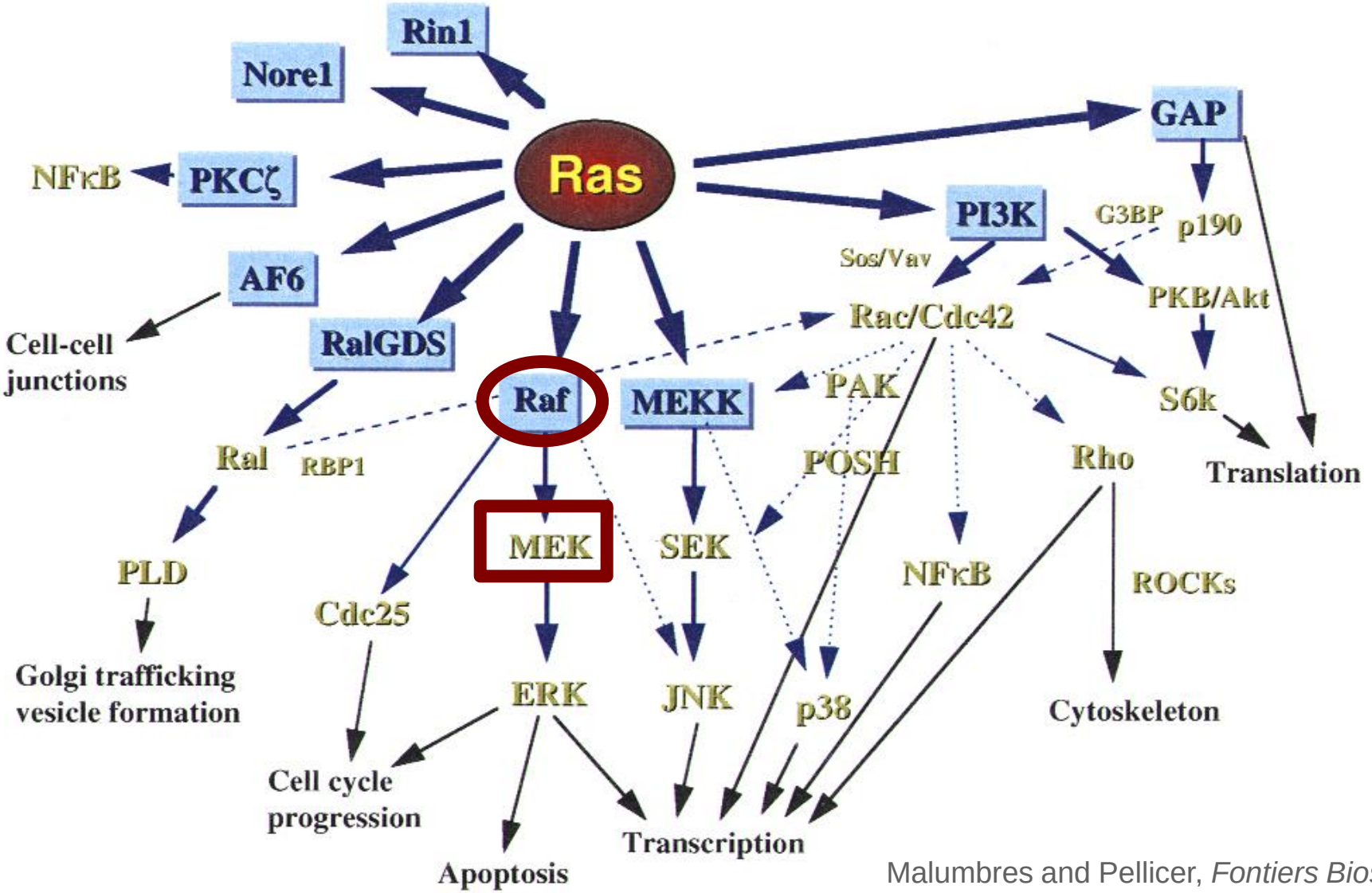
	Number	No tumour response	Tumour response	p value*
Total	34	27 (79%)	7 (21%)	
BRAF mutation				
No	32	25 (78%)	7 (22%)	1·000
Yes	2	2 (100%)	0	
KRAS mutation				
No	20	15 (75%)	5 (25%)	0·672
Yes	14	12 (86%)	2 (14%)	
BRAF or *KRAS* mutation				
No	18	13 (72%)	5 (28%)	0·405
Yes	16	14 (88%)	2 (13%)	

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

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

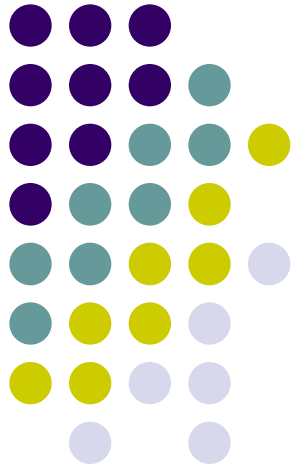
Farley, *Lancet Oncol* 2013

RAS signaling



Malumbres and Pellicer, *Fontiers Biosci* 1998

High grade serous



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

The Cancer Genome Atlas, *Nature* 2011

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).

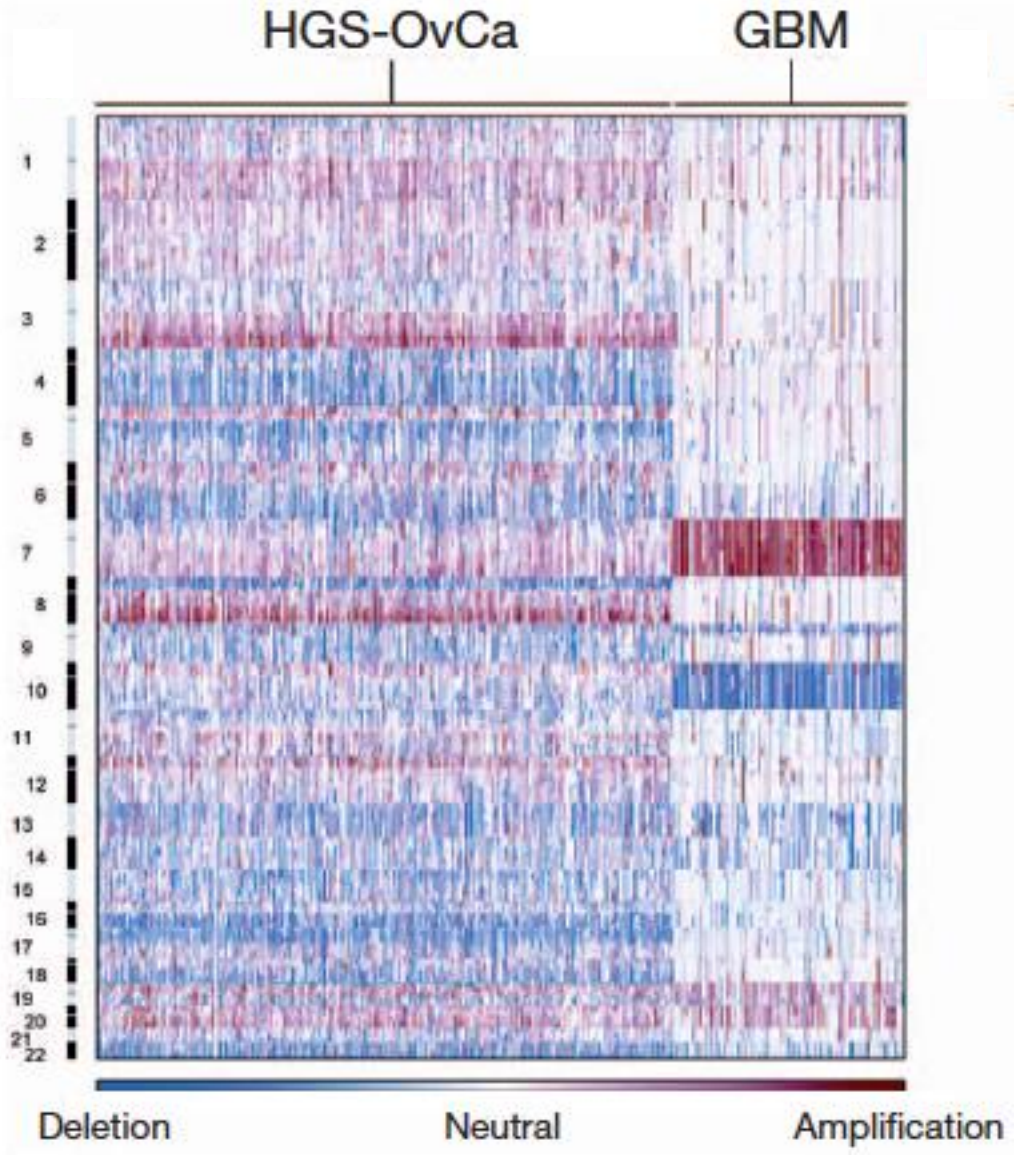


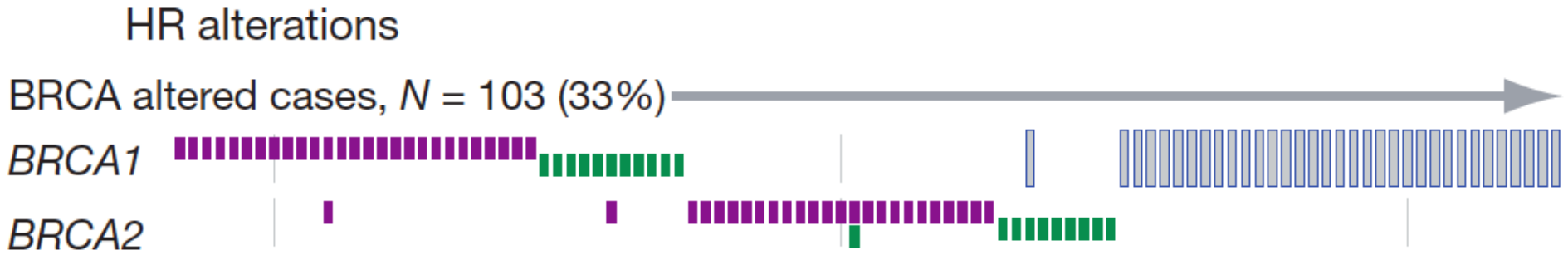
Table 2 | Significantly mutated genes in HGS-OvCa

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

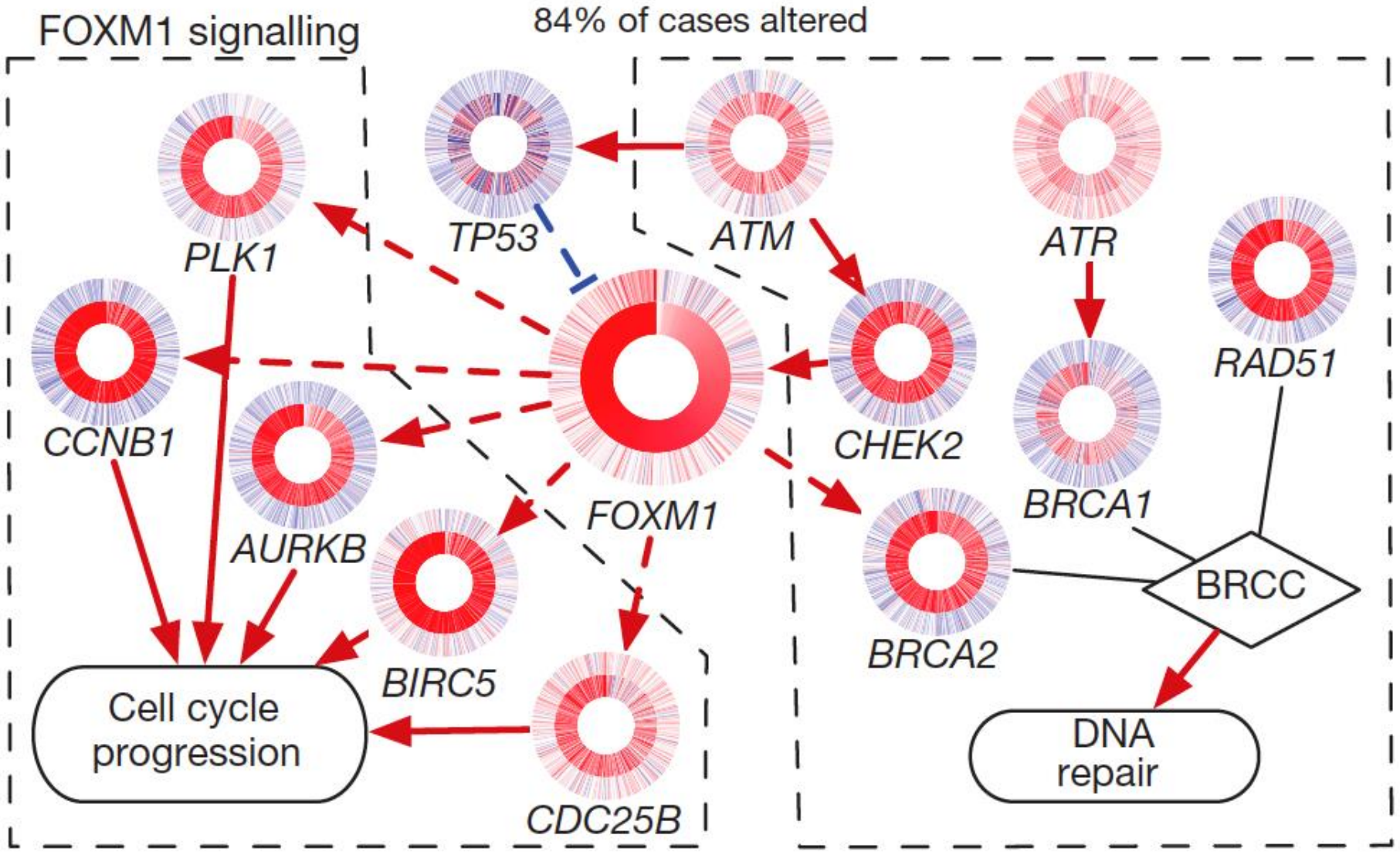
Validated mutations are those that have been confirmed with an independent assay. Most of them are validated using a second independent whole-genome-amplification sample from the same tumour. Unvalidated mutations have not been independently confirmed but have a high likelihood to be true mutations.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

The Cancer Genome Atlas, *Nature* 2011

Altered pathways in HGS-OvCa

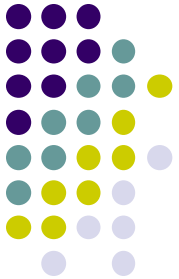


Altered pathways in HGS-OvCa



The Cancer Genome Atlas, *Nature* 2011

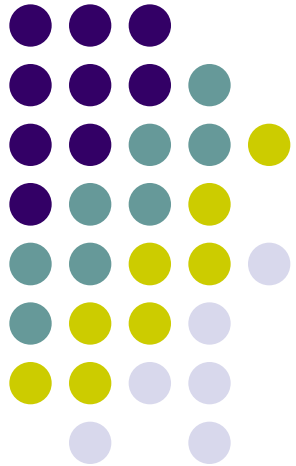
TCGA – what next?



- 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 ...

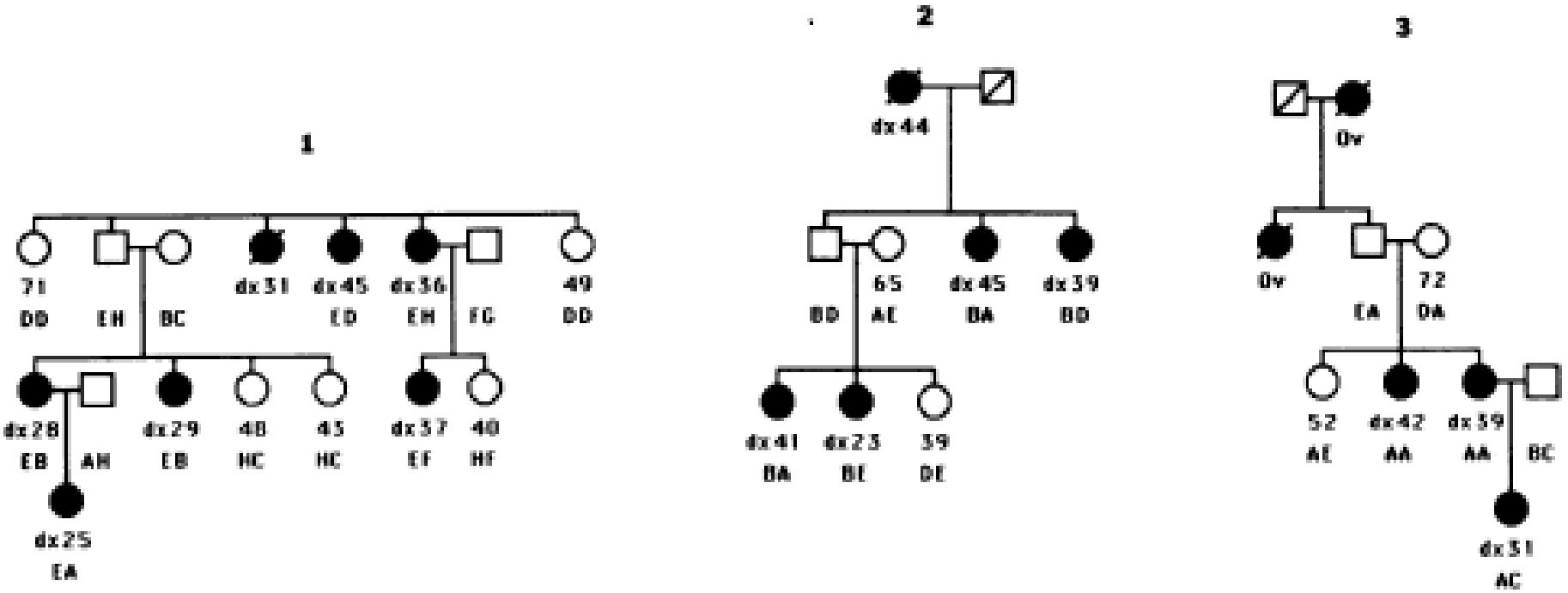
Targeting deficient Homologous Recombination

PARP inhibitors

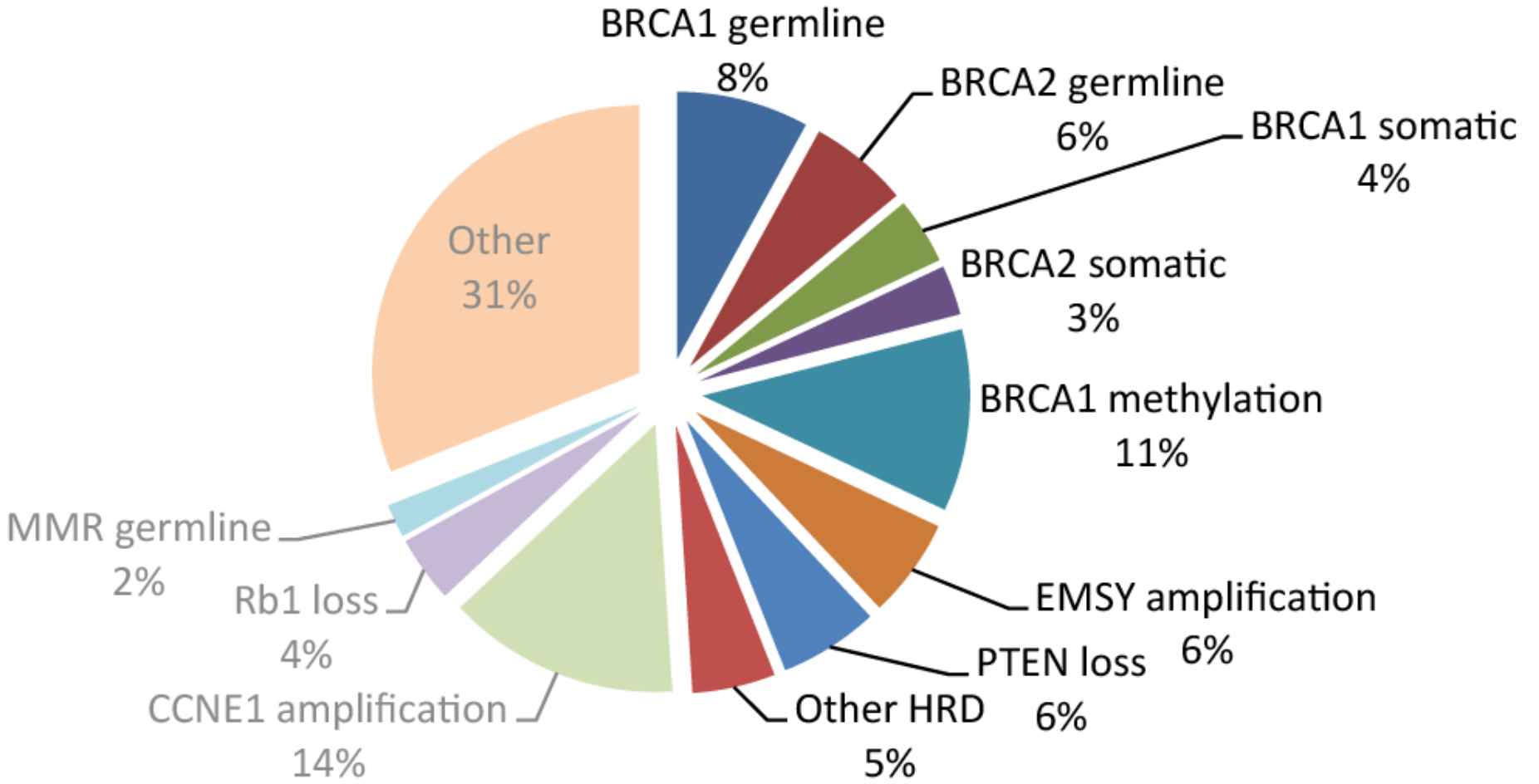


BRCA mutations

- Hall…King, *Science*, 1990



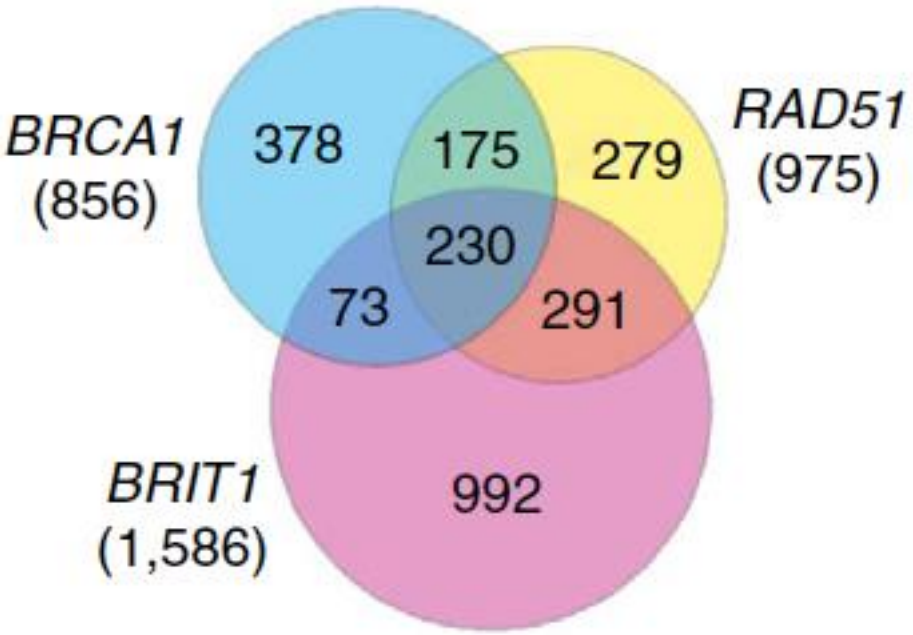
High grade serous cancers

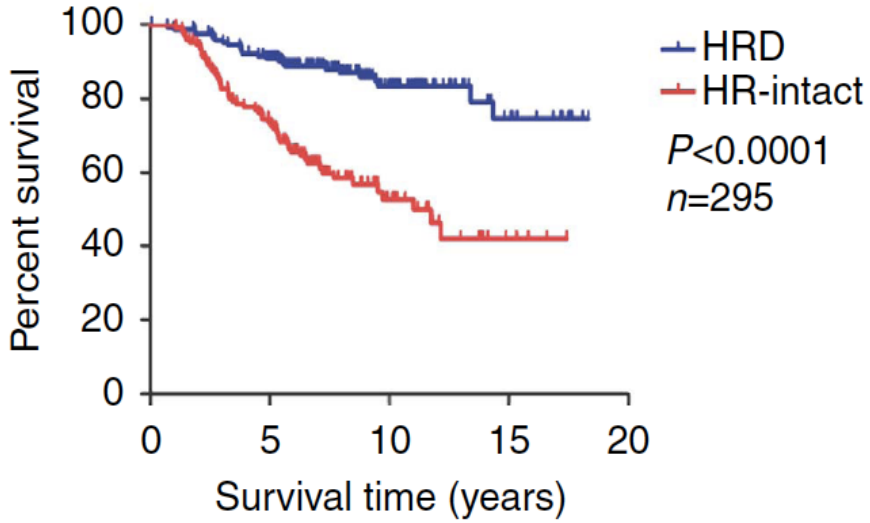


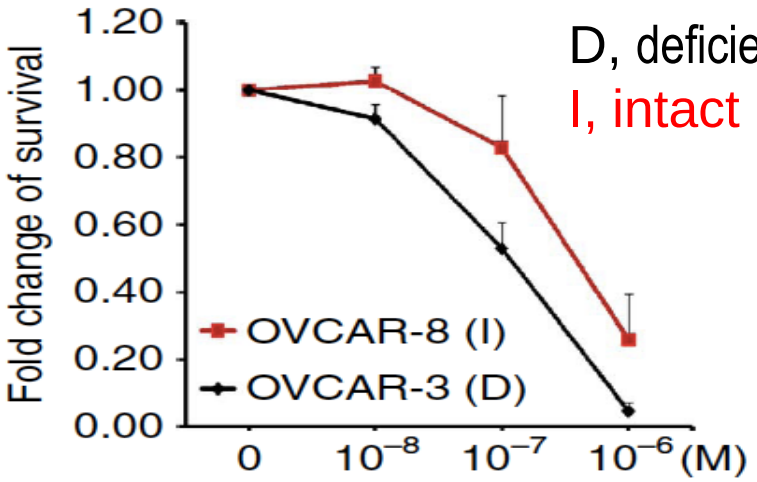
* HRD, homologous recombination defect

BRCA mutations… and beyond

Genes associated with mutations in Homolgous Recombination machinery







Peng et al, *Nat Comm,* 2014

PARP inhibition: BRCA-mutant cancers

cellular metabolism, environmental exposures

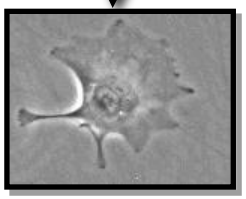
inhibitor

Replicating cells

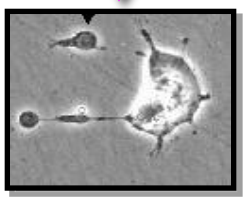
Normal cell

Repair by Homologous Recombination

Survival



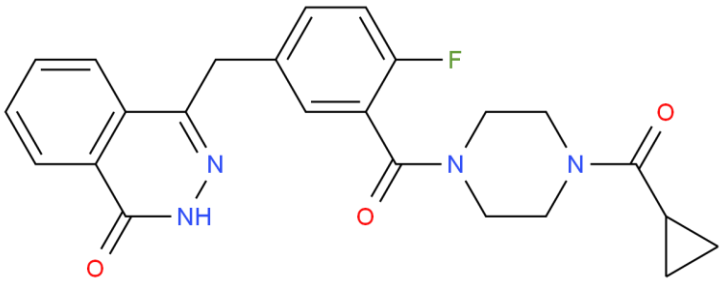
Cancer cell with BRCA deficiency

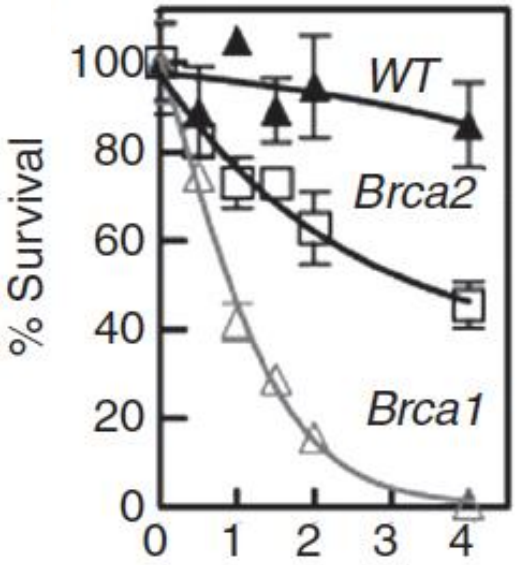


No effective repair (No HR pathway)

PARP inhibitor

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





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)**

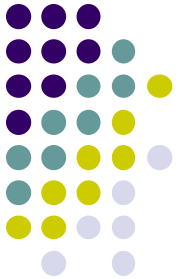
Cohort 3

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



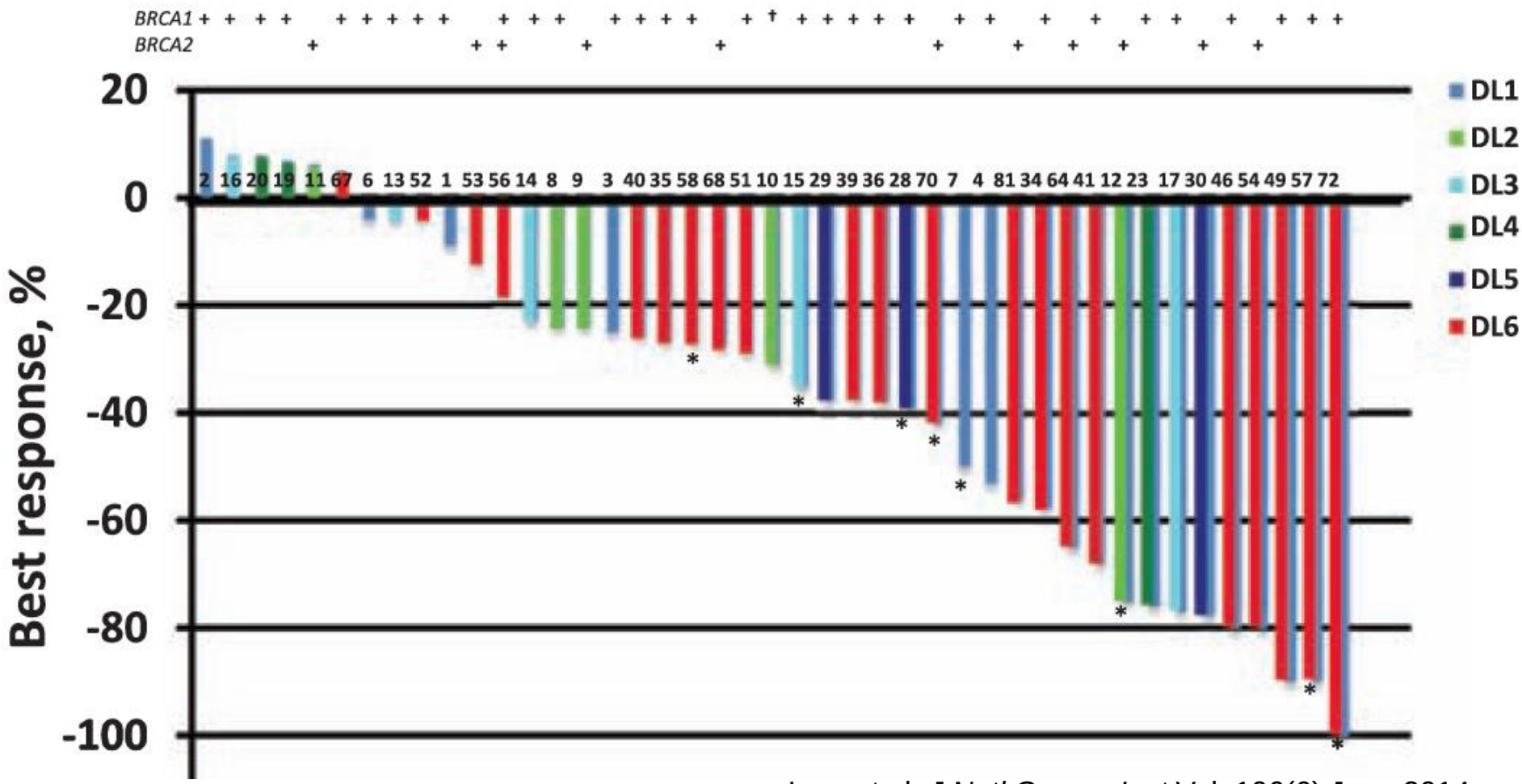
- **Results:** 45 enrolled patients
  - 37 ovarian cancer
  - 8 breast cancer

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



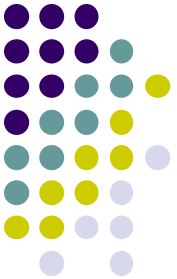
Lee, et al. *J Natl Cancer Inst* Vol. 106(6) June 2014

Phase 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

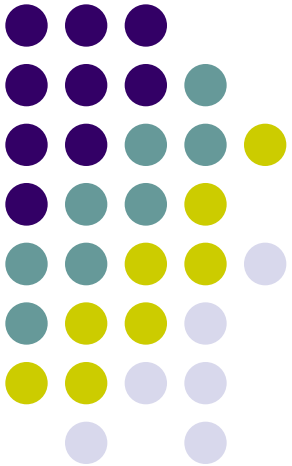


- **Conclusions:**
  - Oral olaparib is well tolerated in combination with carboplatin
  - Highly active in advanced, chemotherapy-refractory BRCA-deficient cancer
  - Greater activity seen at the higher dose
  - Positive proof of the concept of the activity and tolerability of **genetically defined 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.”

Redig and Janne, *J Clin Oncol* 2015

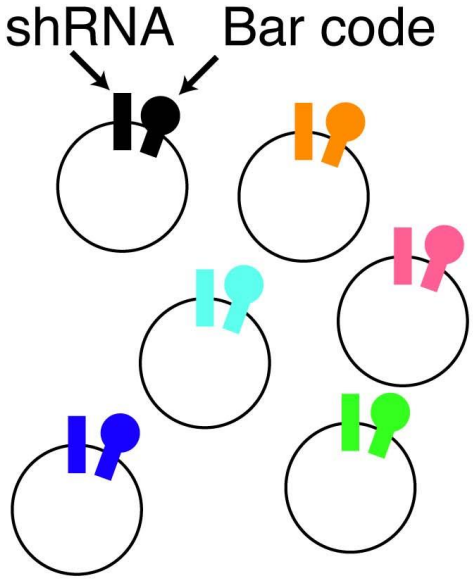
“Actionable” targets

- Need a functional experiment

- Functional genomics

Using a functional genomics screen to identify targets

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

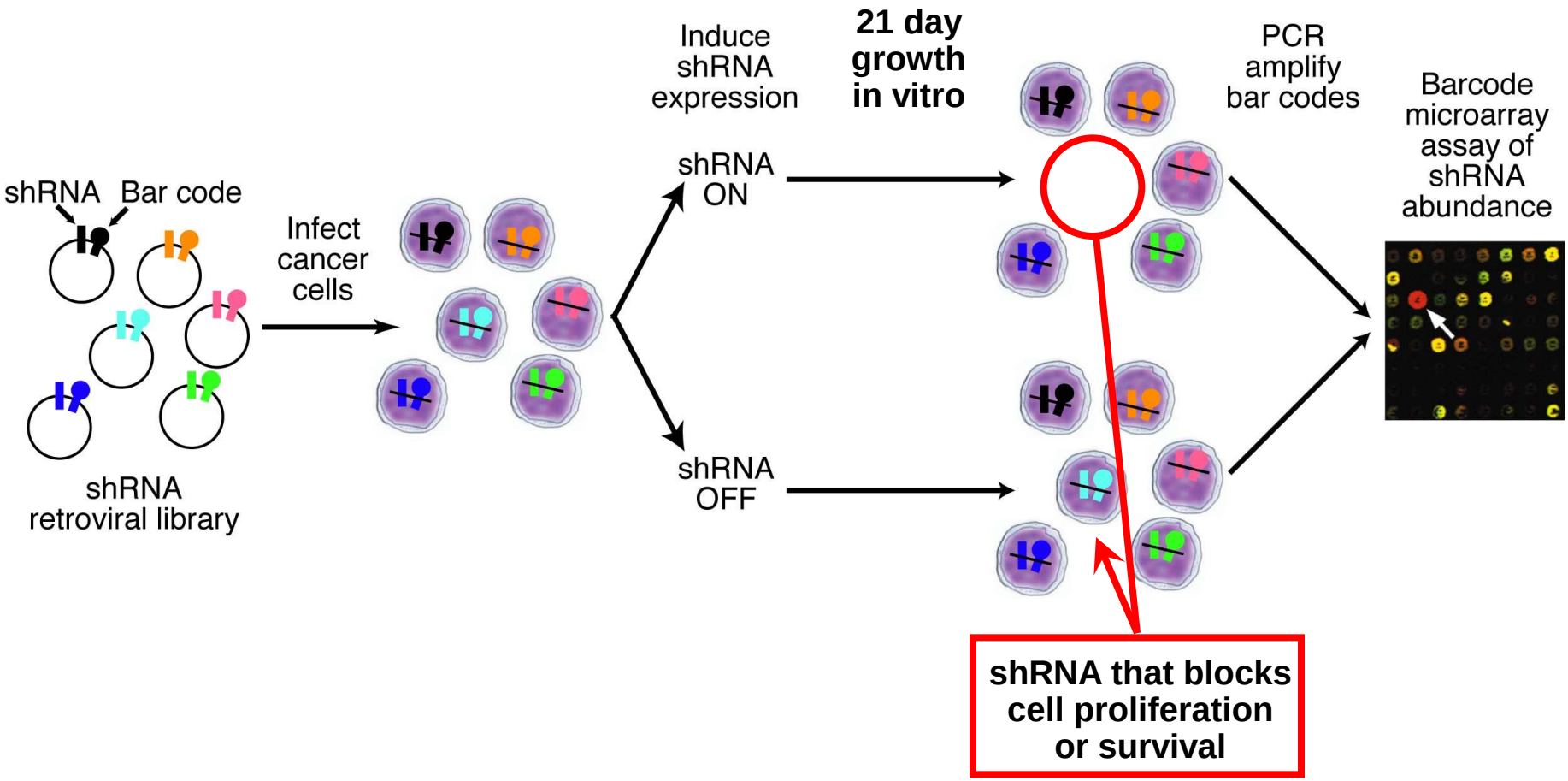


shRNA retroviral library

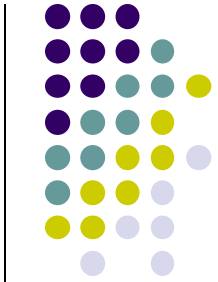
-- shRNAs targeting **2500** human genes
-- 3 shRNA constructs per gene
-- All sequence verified
-- All containing identified 60-mer bar code sequence
-- shRNA expression is inducible by doxycycline
-- Library target genes:
- All protein kinases
- All PI3 kinase
- All deubiquitinating enzymes
- NF-kB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

Ngo, et al. *Nature* 2006

shRNA Library Screen for Genes Controlling Cancer Cell Proliferation and Survival

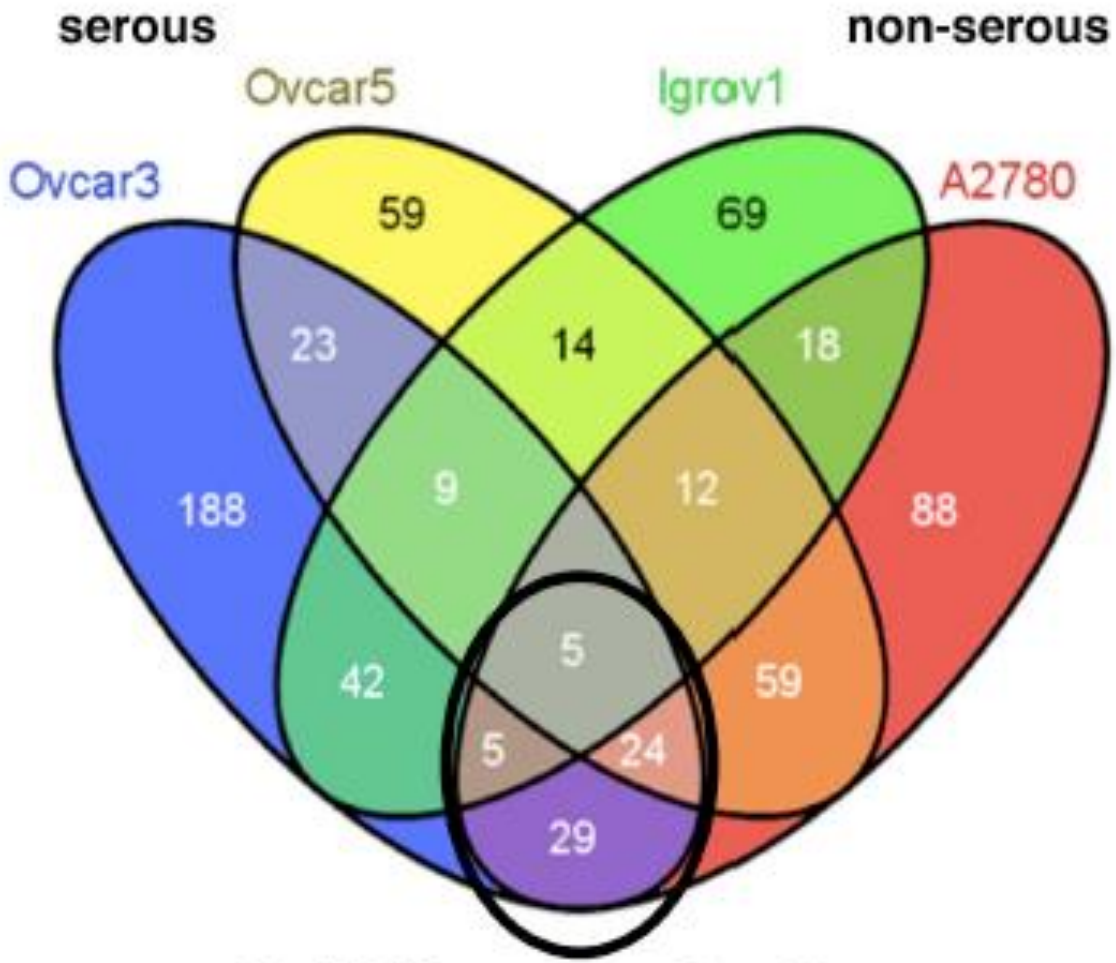


Functional Genomics of ovarian cancer



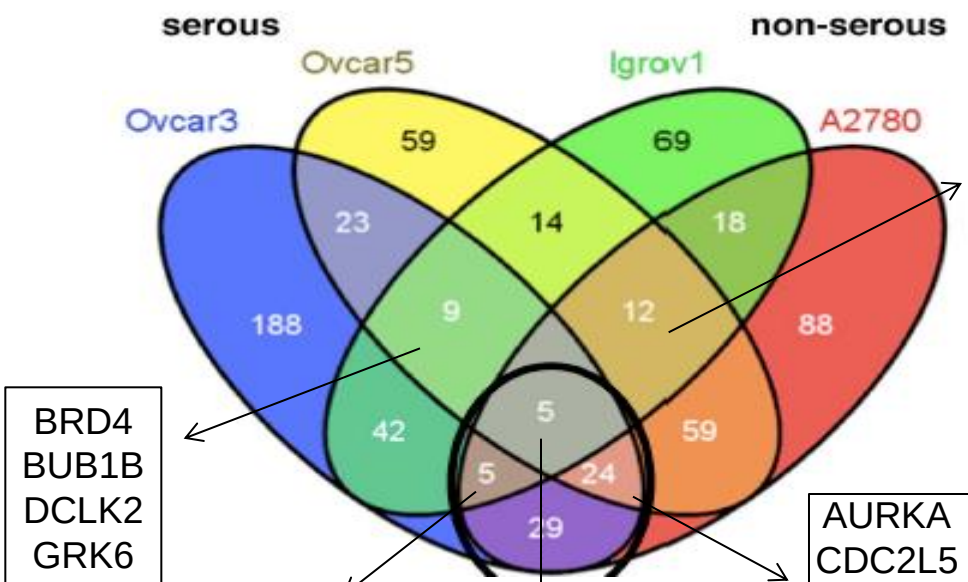
- Four ovarian cancer cell lines
  - OVCAR3 – serous
  - OVCAR5 – serous
  - Igrov1 – non-serous
  - A2780 – non-serous

Common targets in ovarian cancer – “drivers”?



63 shRNAs representing 55 genes

Common targets in ovarian cancer – “drivers”?



ALPK2
CDC2L6
CDK7
CSNK12
ERBB2
FER
KSR2
MAP3K8
NEK2
RIPK5
TLK1
WNK1

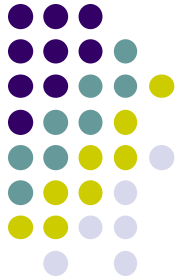
BRD4
BUB1B
DCLK2
GRK6
ITK
PDGFRB
RET
SGK2
STK36

DDR2
ERN2
INSRR
MAP2K7
RRM1

GUCY2F
MKNK2
PDK3
PIK3AP1
WEE1

AURKA	MGC42105
CDC2L5	NLK
CDC7	NUAK1
DCLK3	PLK1
EPHB1	PNCK
FGR	PRKCA
GSK3A	PRKCB
HIPK4	STK32A
KSR1	TAOK1
LRRK2	TEK
MAP3K7	TRRAP
MARK3	TSSK3

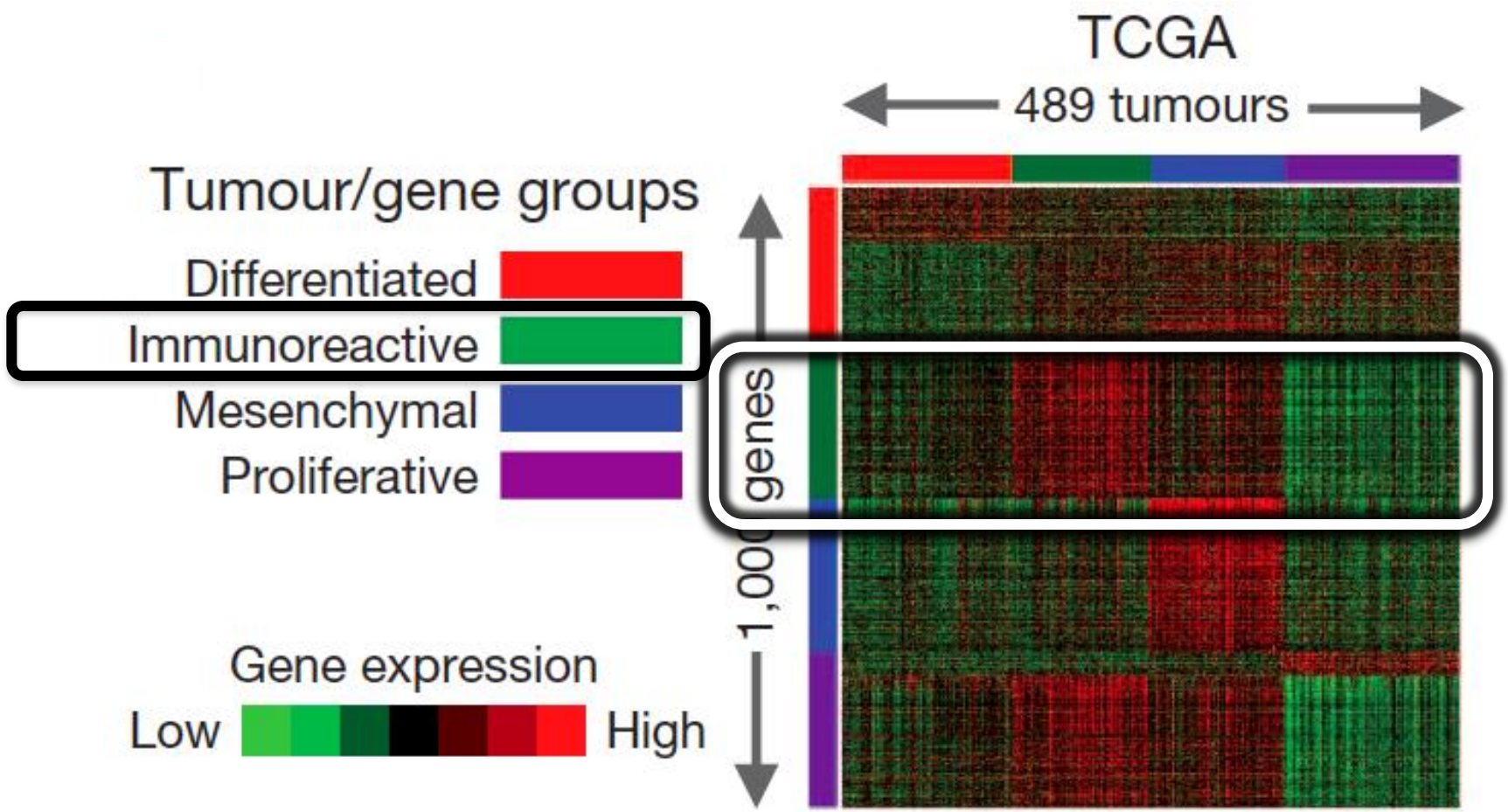
Functional genomics of ovarian cancer



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

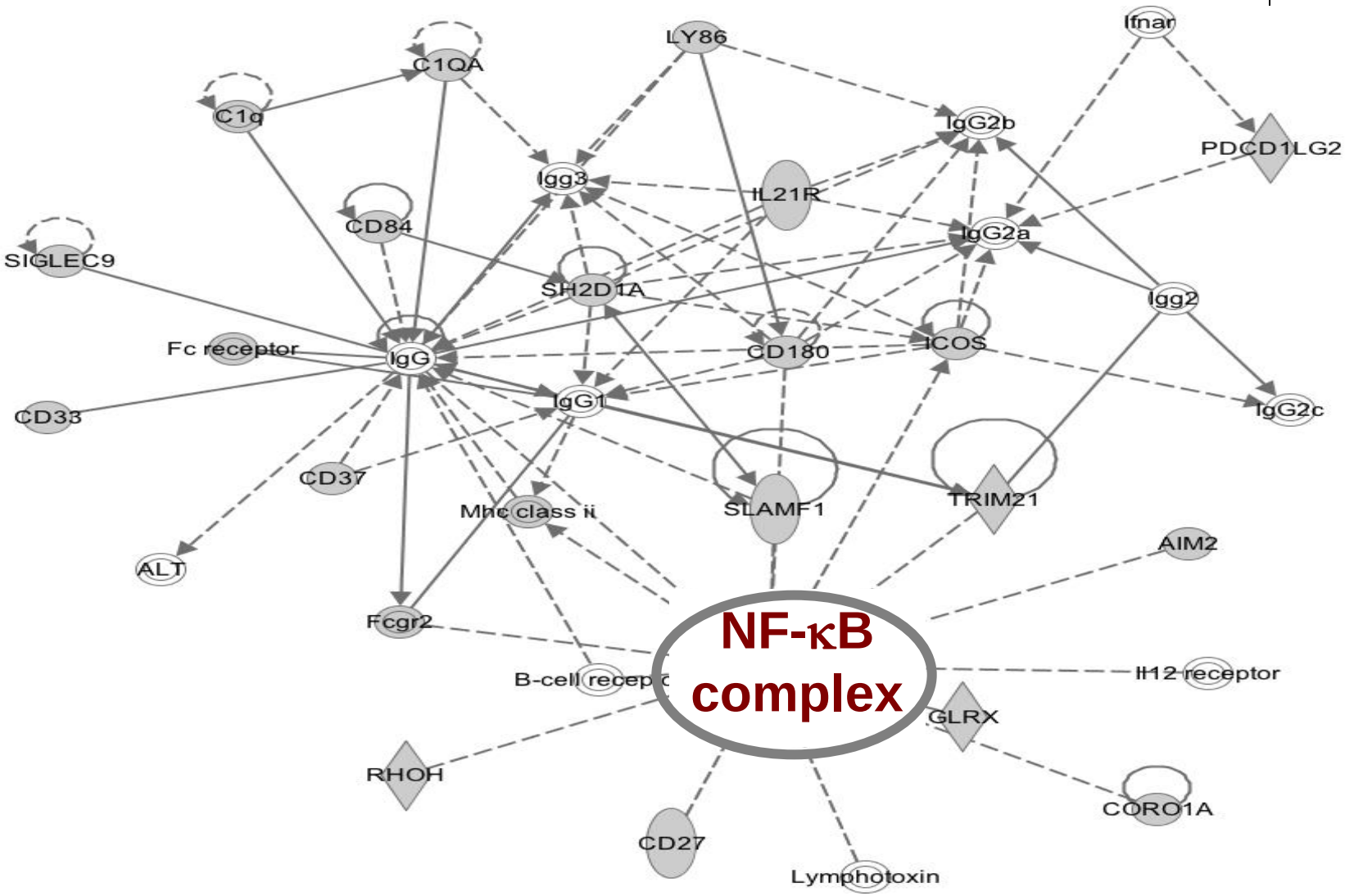
- Focused functional screens
  - Specific subgroup of serous ovarian cancer
  - NF-kappaB signaling pathway

Gene expression – subgroups

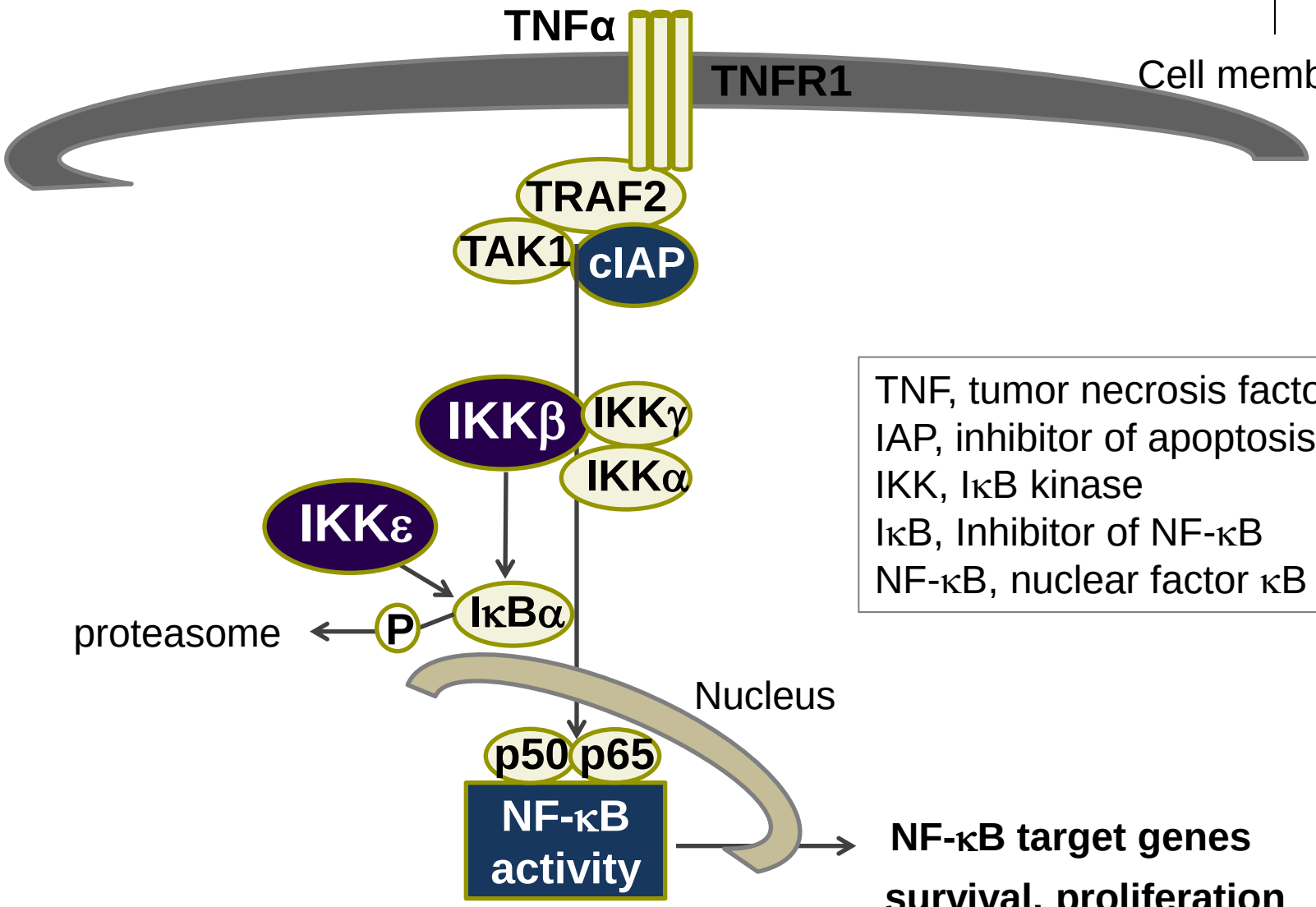


The Cancer Genome Atlas, Nature 2011

Gene expression – immunoreactive

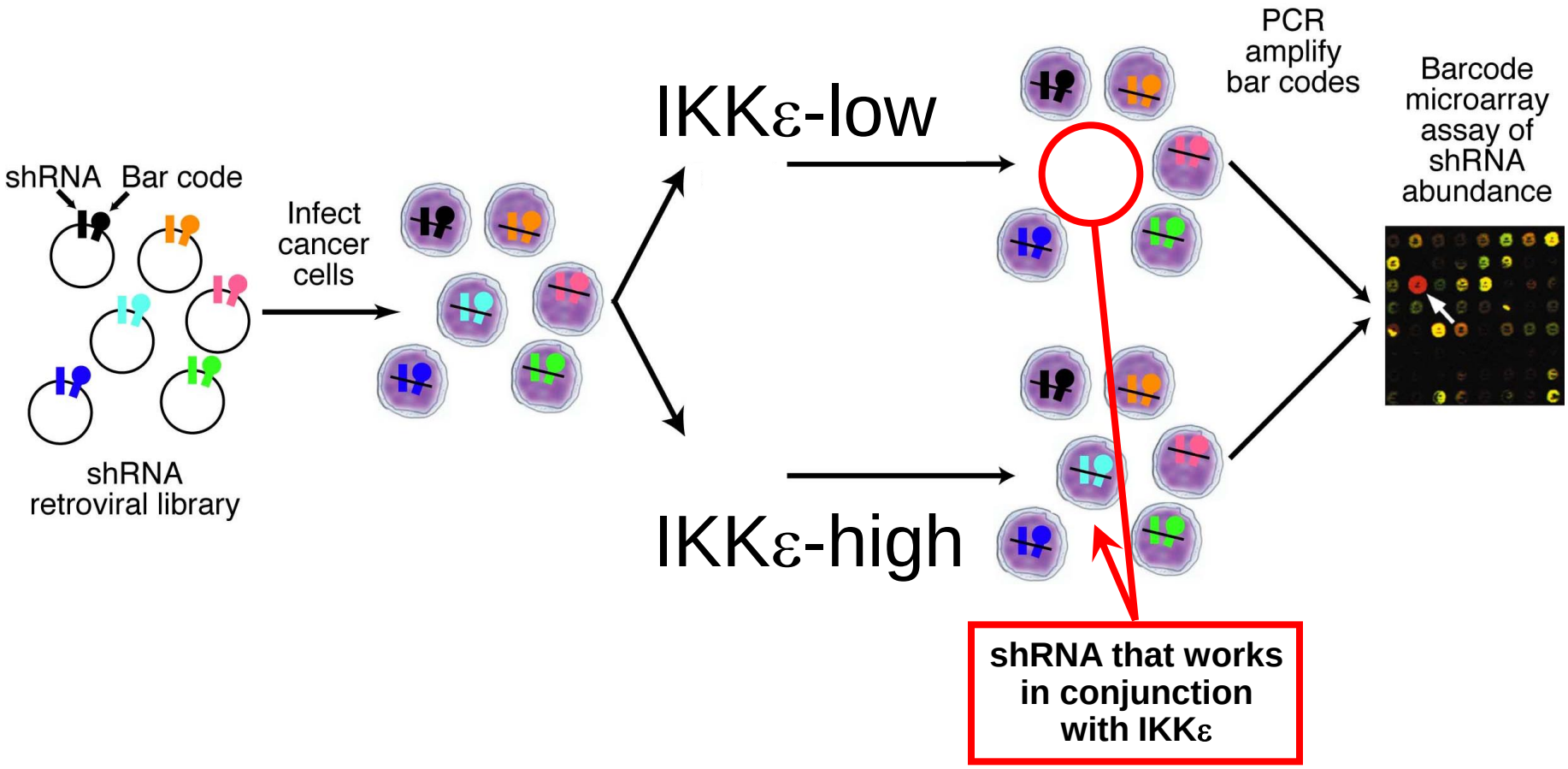


NF-κB signaling



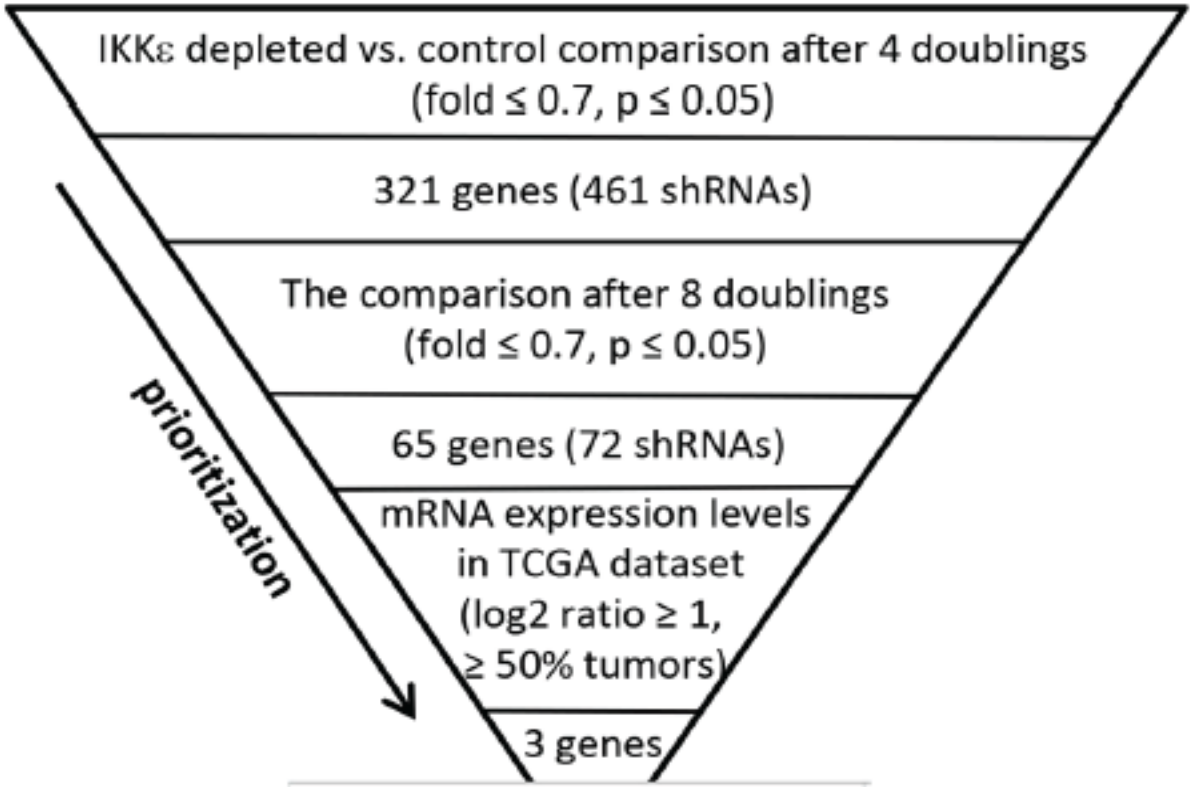
TNF, tumor necrosis factor
IAP, inhibitor of apoptosis protein
IKK, IκB kinase
IκB, Inhibitor of NF-κB
NF-κB, nuclear factor κB

IKKε related targets



CHEK1

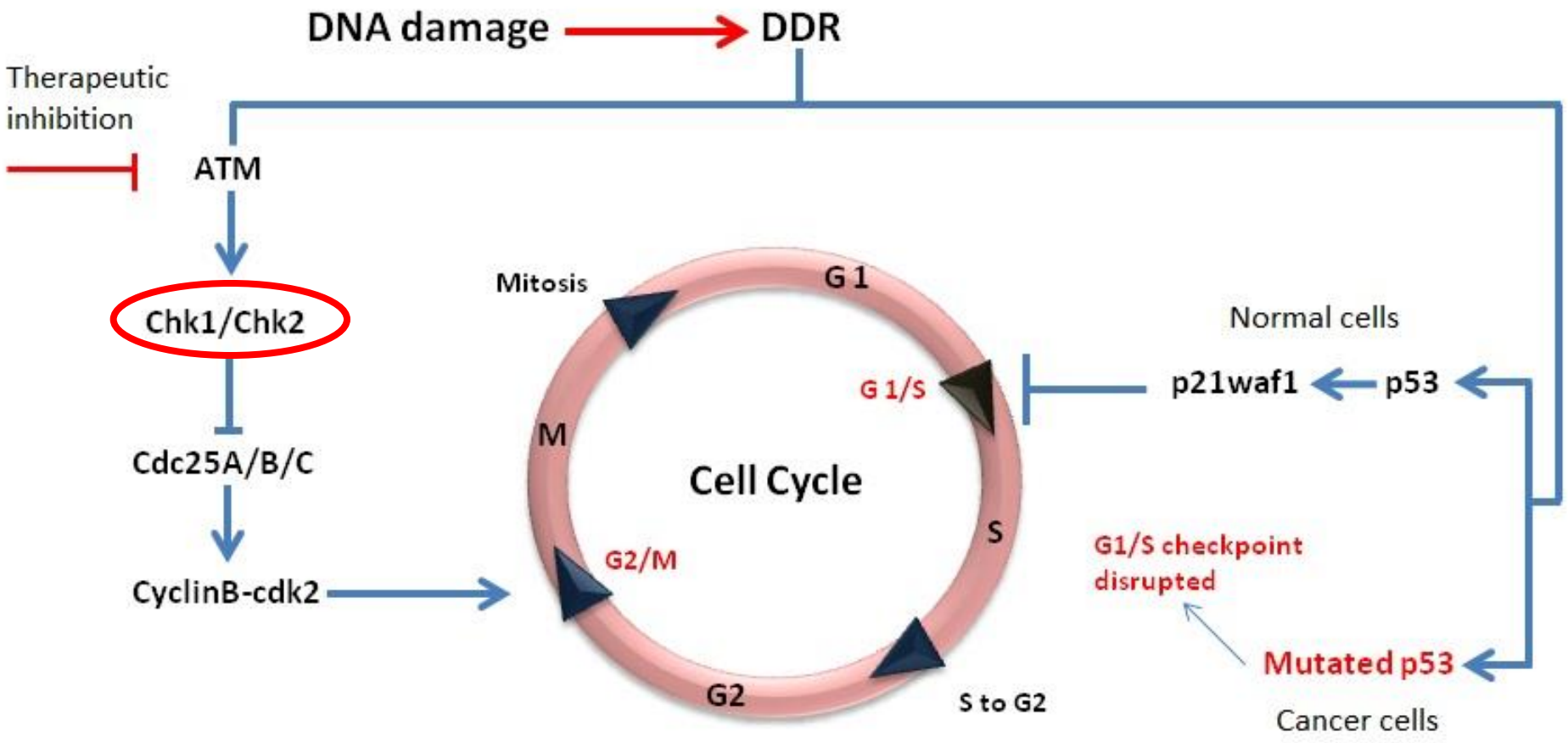
- Highly synergistic with IKKε
- Over-expressed in nearly all ovarian cancers



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

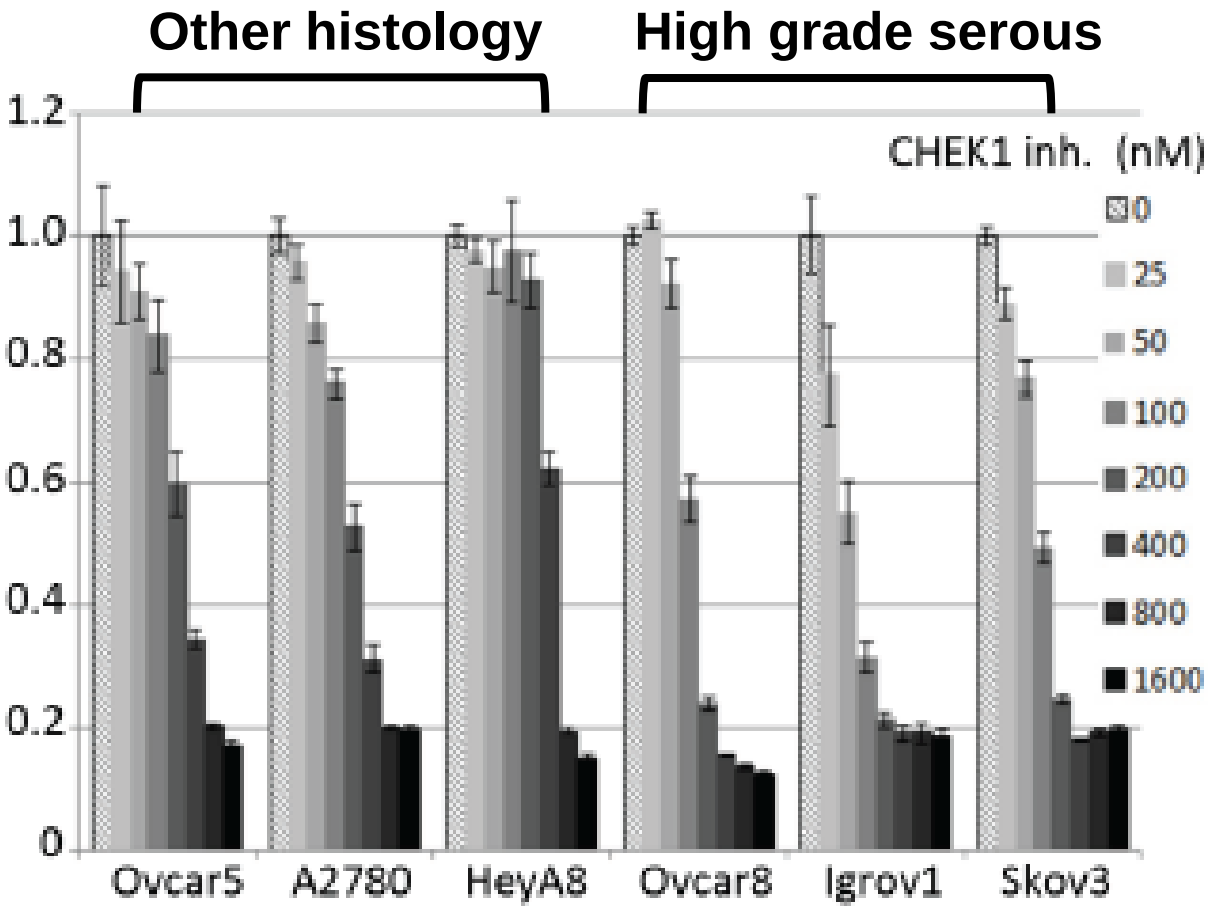
Kim, et al. *Oncotarget*, 2014

CHEK signaling



CHEK inhibitor

- Most potent in HGSOC



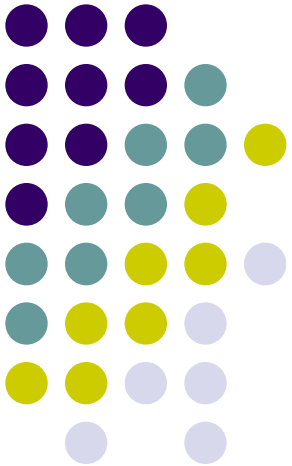
Kim, et al. *Oncotarget*, 2014

CHEK inhibitor

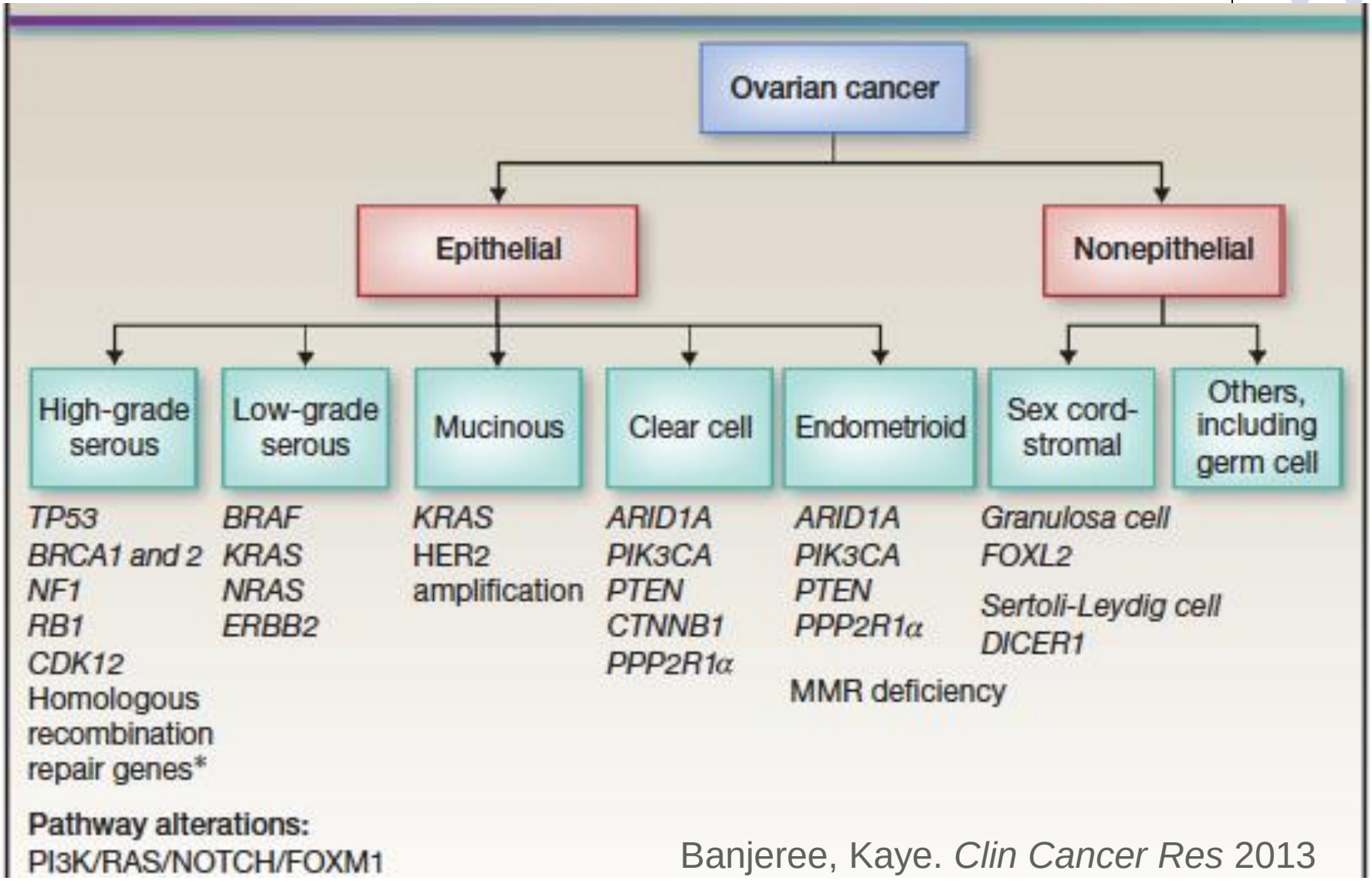
- Clinical trial ongoing
  - NCT02203513
  - Promising results in High grade serous non BRCA
  - Highlighted by a Functional Genomics approach

Ovarian cancer genomics

Summary



Ovarian cancer genomics



Banjeree, Kaye. *Clin Cancer Res* 2013

Functional Genomics

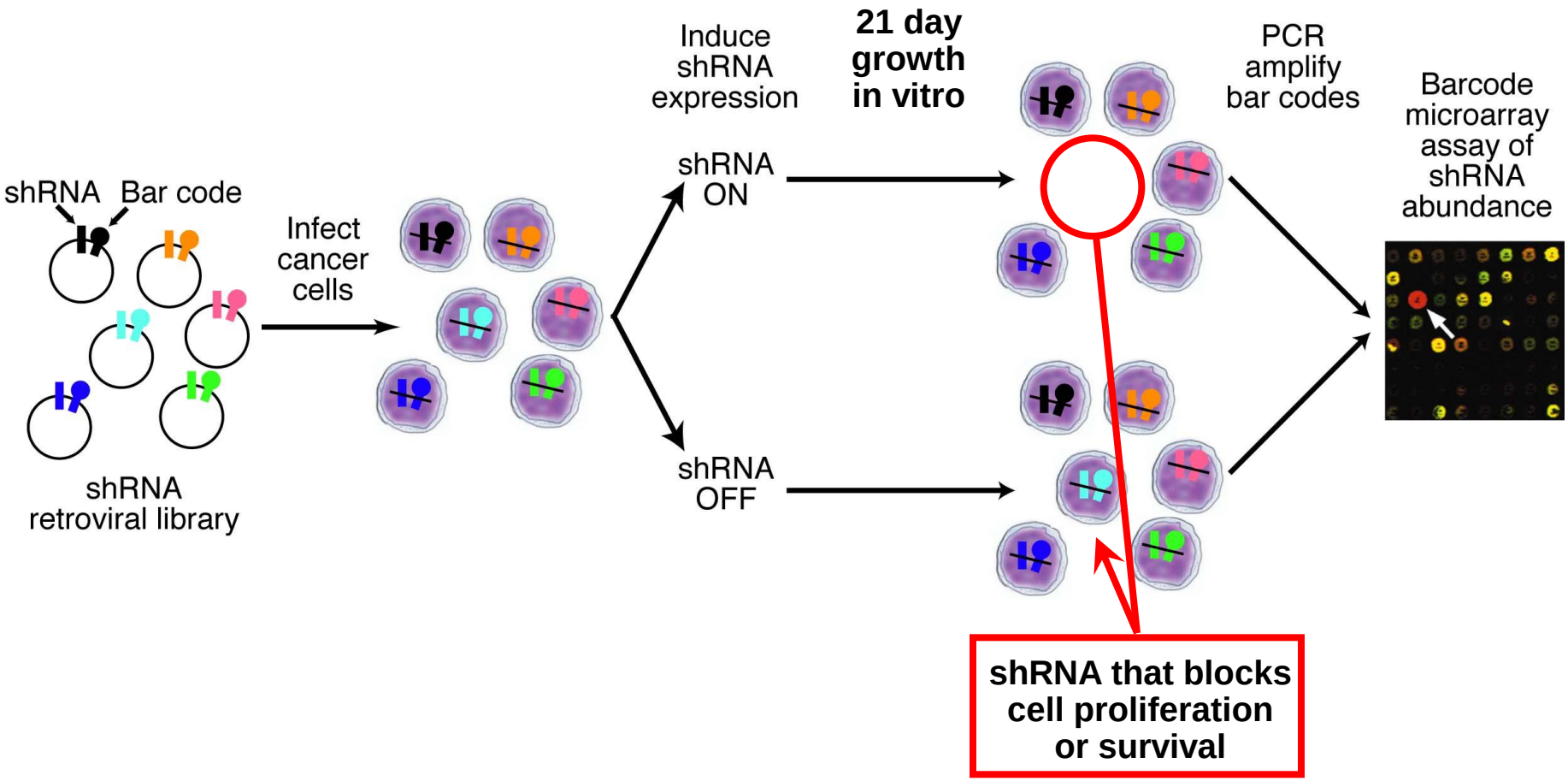
- 1981 – Shih – discovery of Her2/neu

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

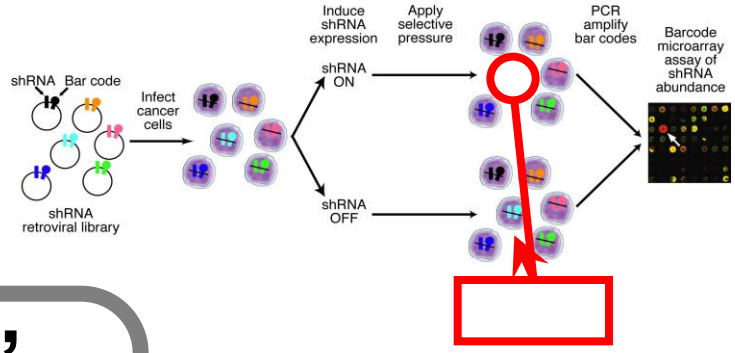
shRNA Library Screen for Genes Controlling Cancer Cell Proliferation and Survival



Ovarian Cancer in the Genomics Era

Functional genomic screen

shRNA Library Screen for Genes Controlling Cancer Phenotypes



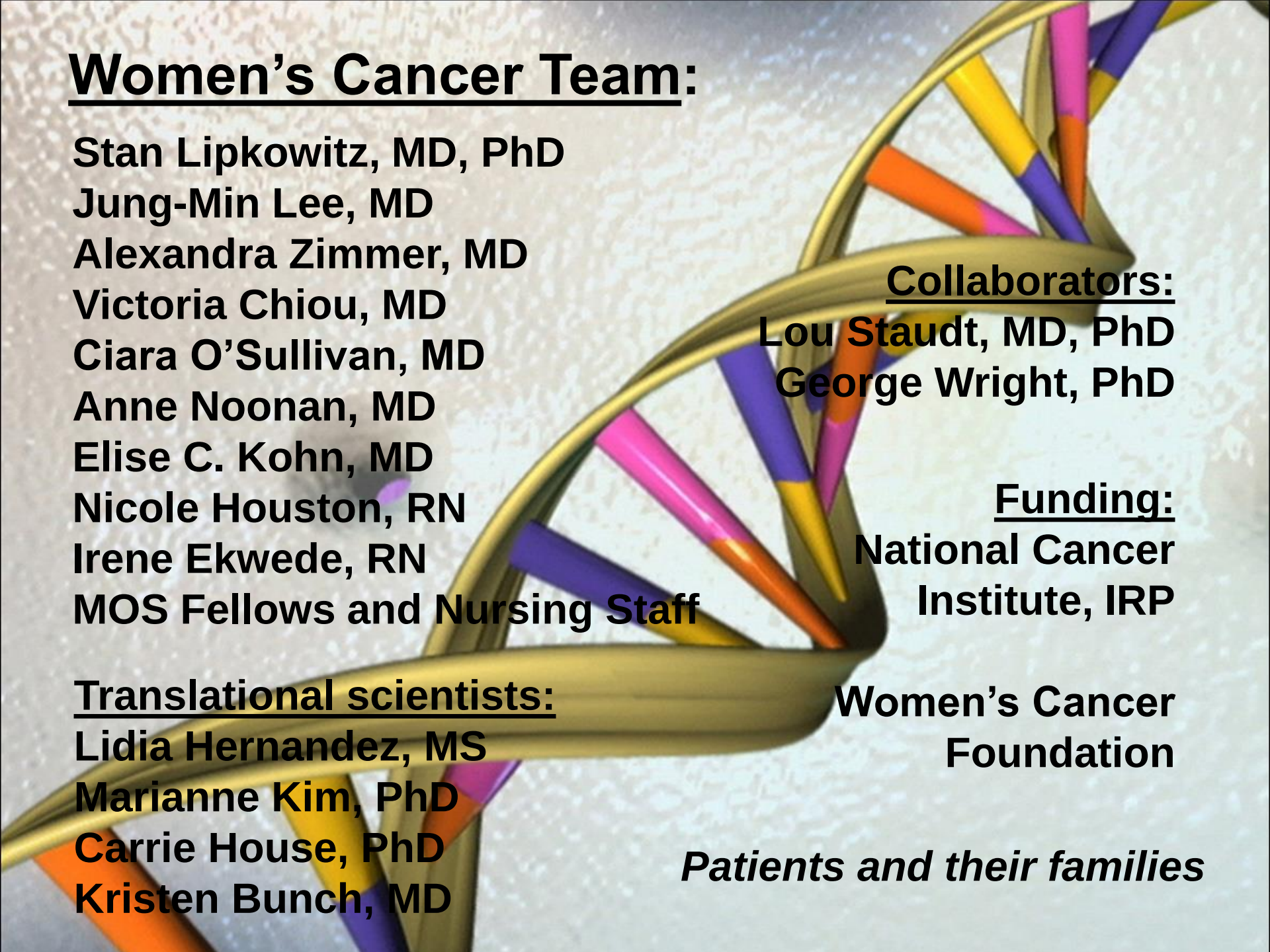
"Driver" aberration/pathway

Clinical trial

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



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George Wright, PhD

Funding:
National Cancer Institute, IRP

Women's Cancer Foundation

Patients and their families