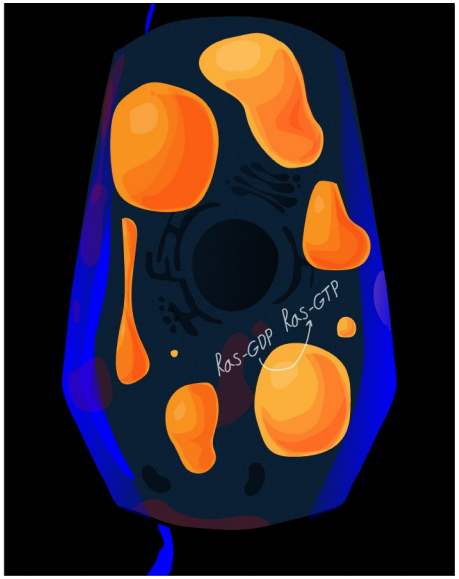
Oncogenes



Asmin Tulpule, MD/PhD

Tow Center for Developmental Oncology
Human Oncology and Pathogenesis Program (HOPP)
Department of Pediatrics

Non-linear Career Path of a Physician-Scientist

"I'm leaving my job so I can spend time with another job."

Cartoon credit: Drew Dernavich

BA: Princeton (1998-2002), Molecular Biology and Biophysics

PhD: Harvard (2002-2008), Human pluripotent stem cells and pediatric bone marrow failure syndromes, Advisor: Dr. George Daley

MD: Harvard (2007-2011)

Pediatric Residency and Hematology/Oncology Fellowship: UCSF with post-doctoral research with Dr. Trever Bivona (2014-2017)

2017-2022: Assistant Professor in Pediatric Hematology/Oncology and started my own lab as part of the UCSF Physician-Scientist Scholar Program

Current: Assistant Member, Tow Center for Developmental Oncology, HOPP, and Pediatrics

What is an oncogene?

What is an oncogene?

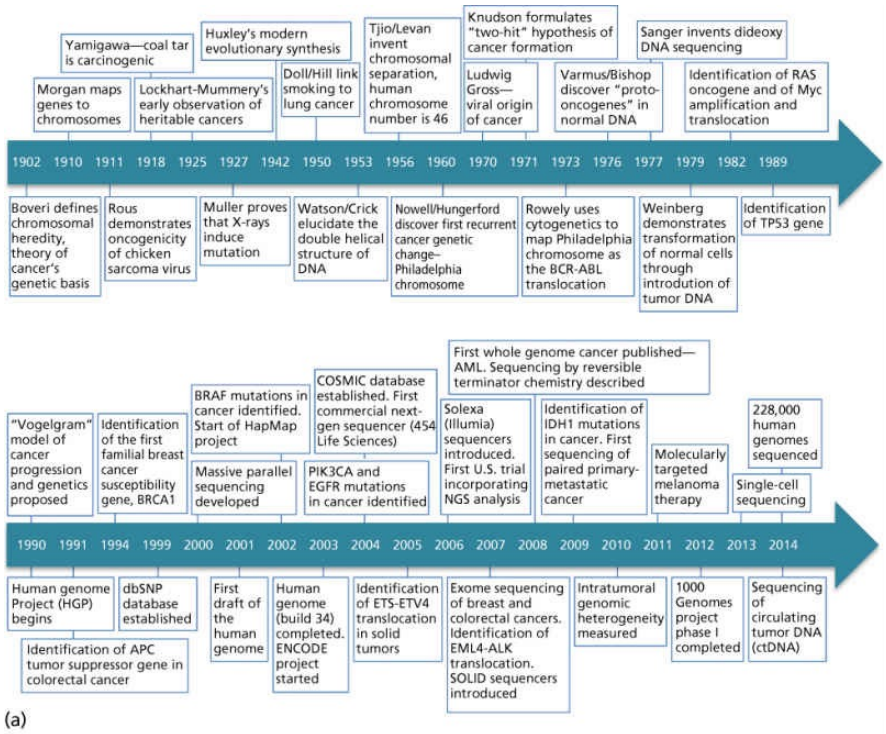
An oncogene is an altered form (mutation, overexpression) of a cellular gene that causes normal cells to become cancerous.

Questions:

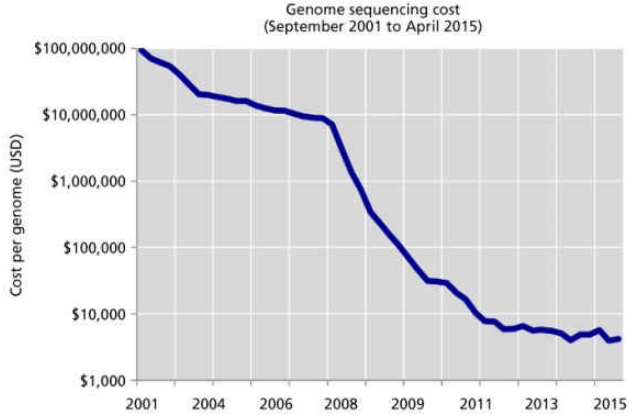
1) How do you distinguish between oncogenes and other types of alterations (tumor suppressor, passenger mutations, polymorphisms)?

2) How do we “prove” that a given oncogene causes cancer?

The genomics revolution in cancer biology

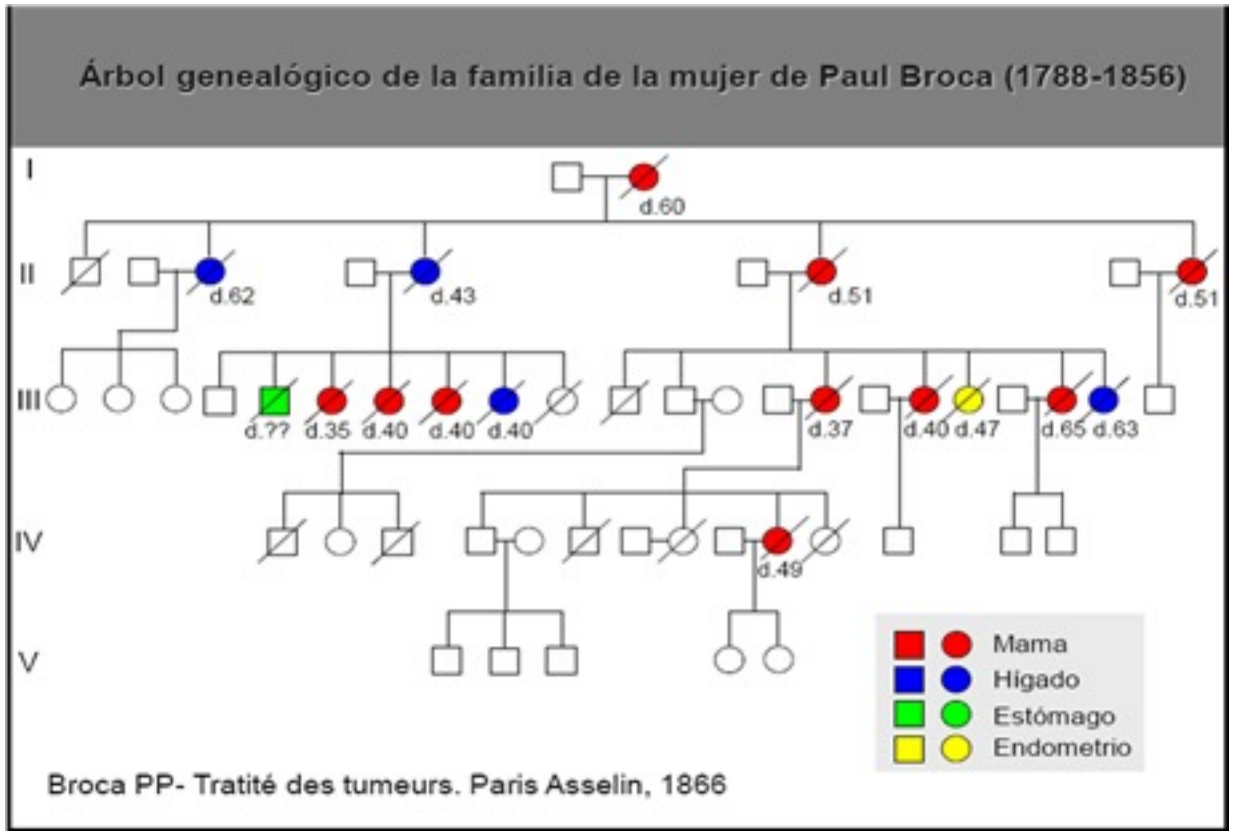


(a)



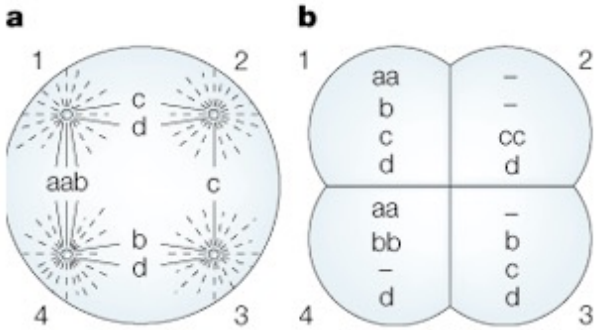
(b)

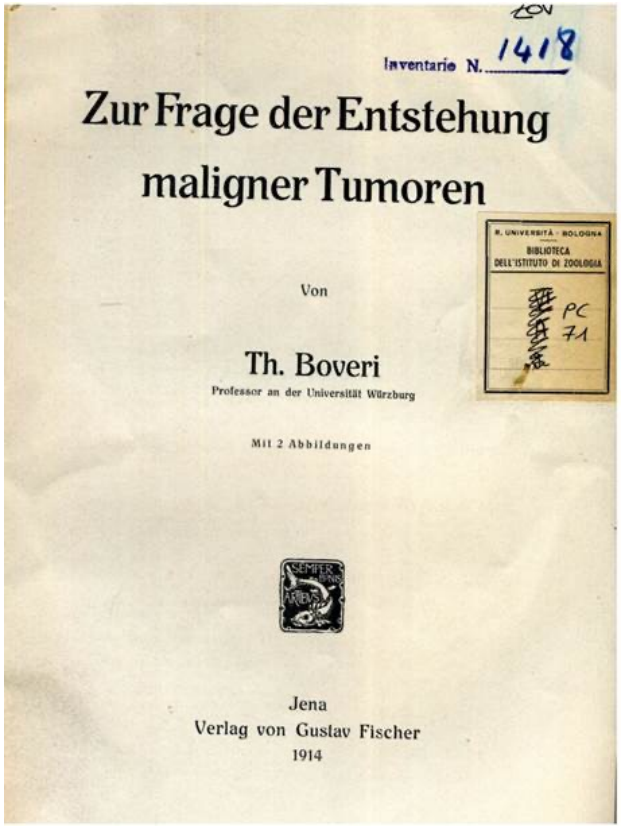
The genetic origins of cancer



1866 – French surgeon Paul Broca described breast CA in a family

Chromosome theory of cancer





Peyton Rous and his chickens

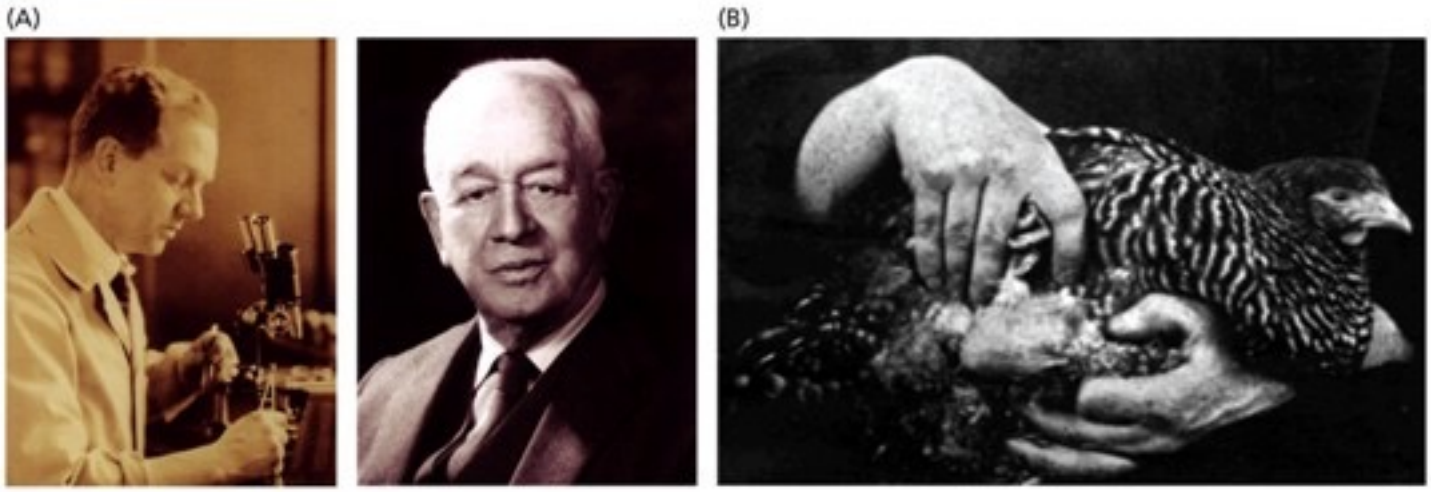
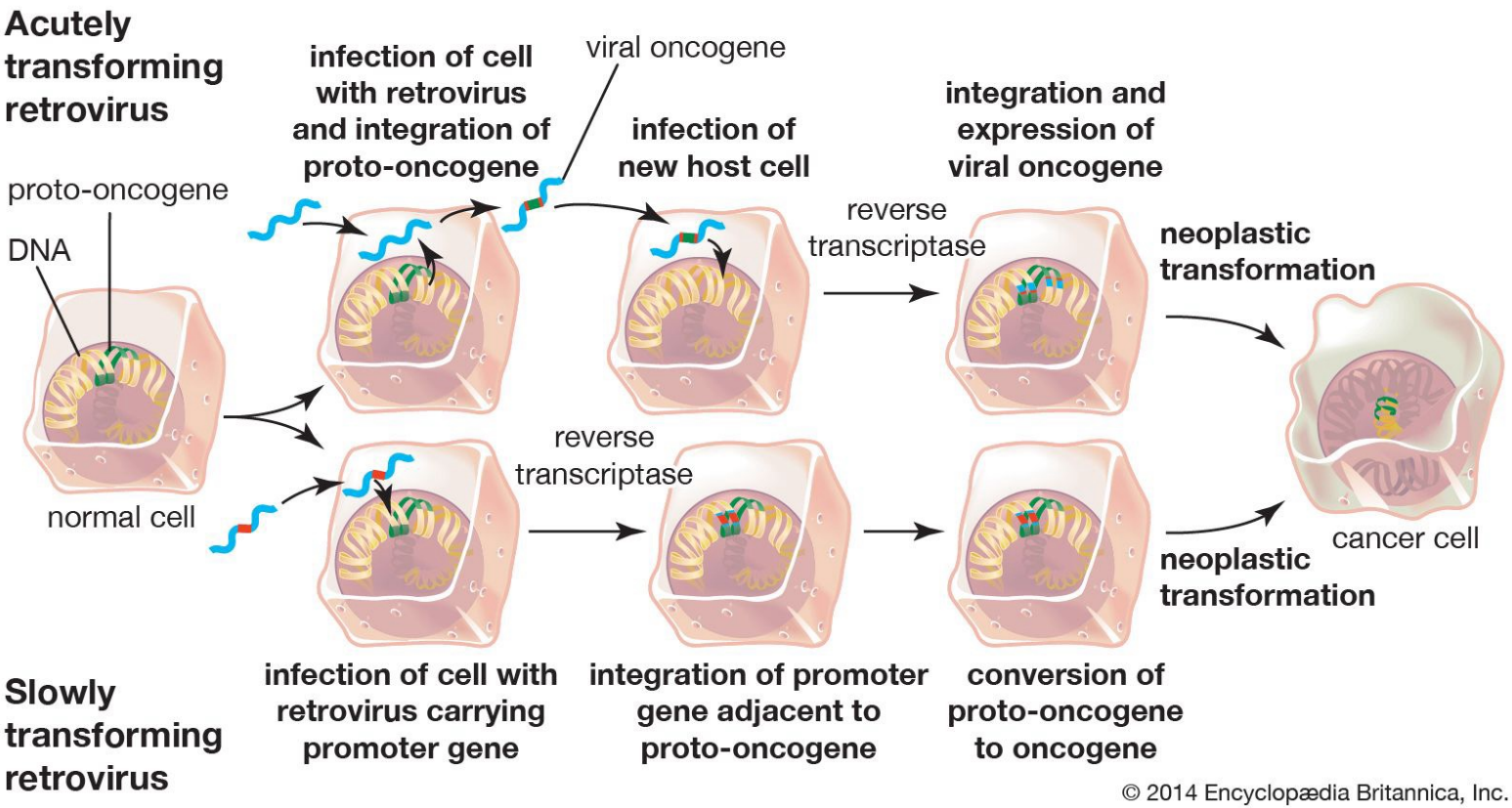


Figure 3.1 The Biology of Cancer (© Garland Science 2014)

Figure 3.2 The Biology of Cancer (© Garland Science 2014)

Retroviral oncogenes?

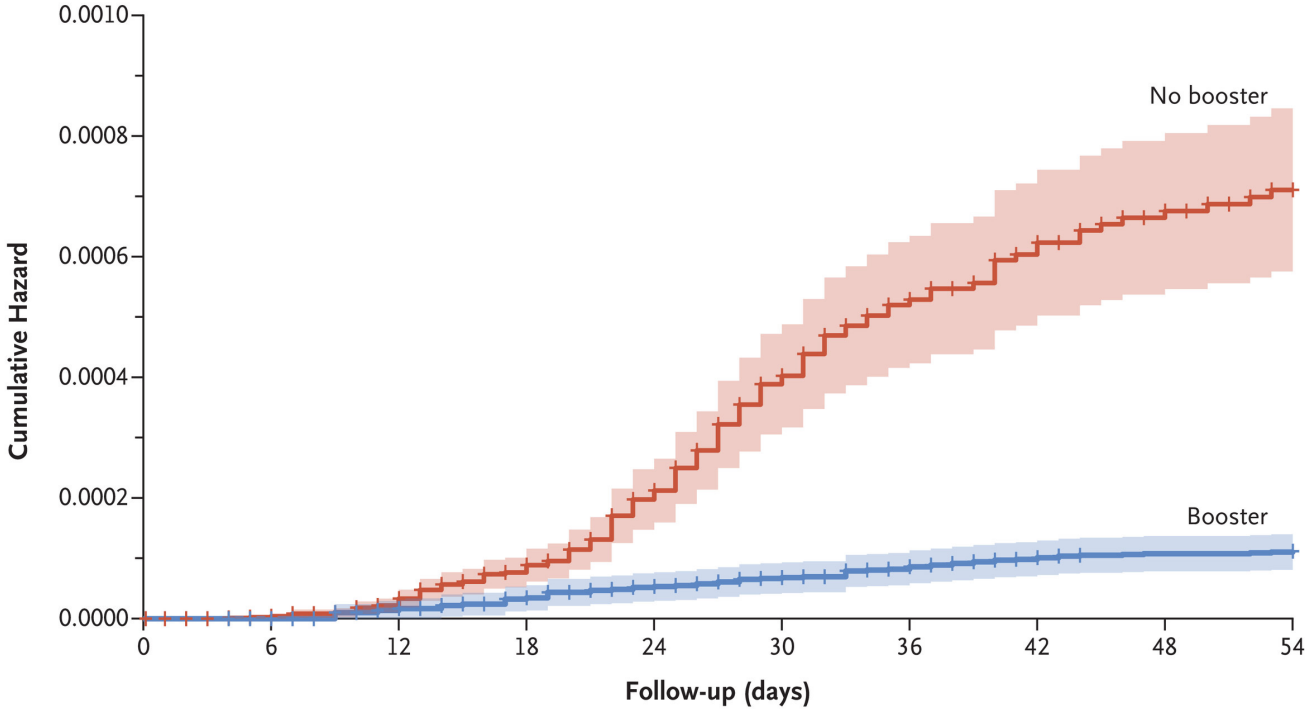


Doll and Hill – smoking and cancer

TABLE IV.—*Proportion of Smokers and Non-smokers in Lung-carcinoma Patients and in Control Patients with Diseases Other Than Cancer*

Disease Group	No. of Non-smokers	No. of Smokers	Probability Test
Males:			
Lung-carcinoma patients (649)	2 (0·3%)	647	P (exact method) = 0·00000064
Control patients with diseases other than cancer (649) . .	27 (4·2%)	622	
Females:			
Lung-carcinoma patients (60)	19 (31·7%)	41	$\chi^2 = 5{\cdot}76$; n = 1 0·01 < P < 0·02
Control patients with diseases other than cancer (60) . .	32 (53·3%)	28	

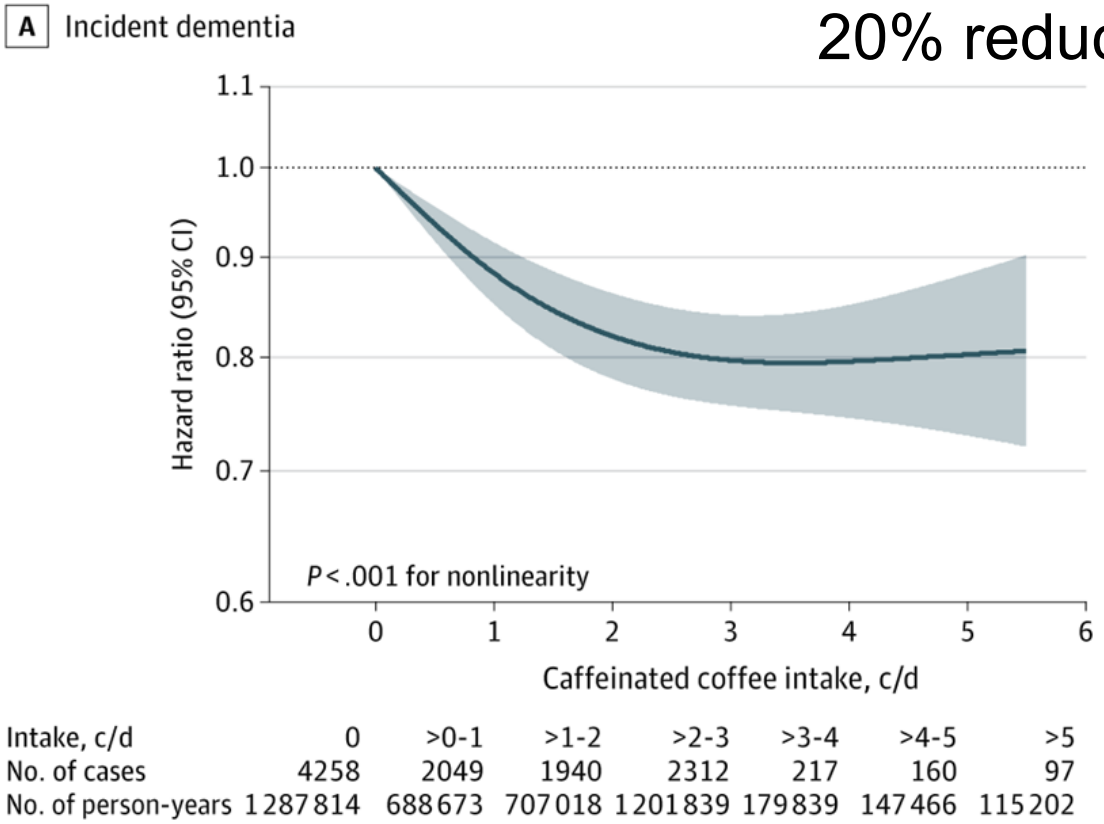
Causality



No. at Risk										
No booster	841,428	723,609	520,459	326,741	202,797	145,021	111,761	101,695	90,036	83,989
Booster	46,259	119,332	322,203	515,639	639,315	696,859	729,971	739,945	756,591	757,614
Cumulative No. of Events										
No booster	0	3	20	43	72	103	119	129	134	137
Booster	0	0	4	12	23	33	46	57	62	65

Causality

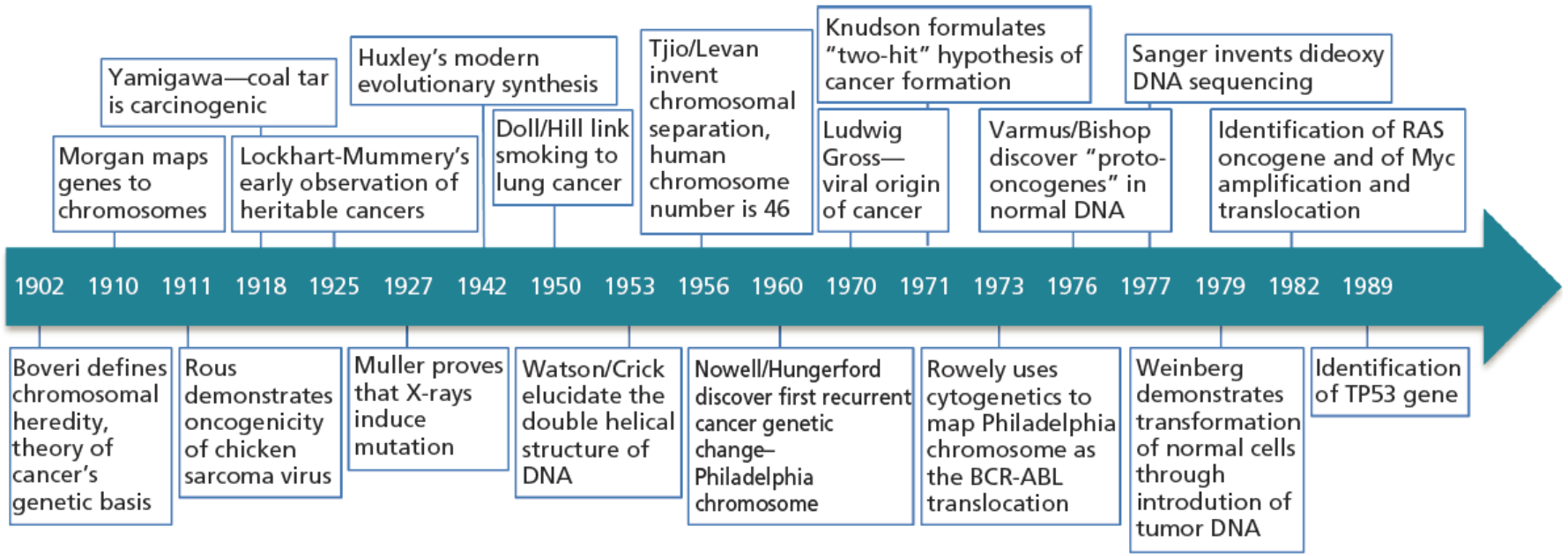
2 cups ->
20% reduction in dementia risk



Intake, c/d	0	>0-1	>1-2	>2-3	>3-4	>4-5	>5
No. of cases	4258	2049	1940	2312	217	160	97
No. of person-years	1287814	688673	707018	1201839	179839	147466	115202

Challenges with observational or case-cohort matching studies:

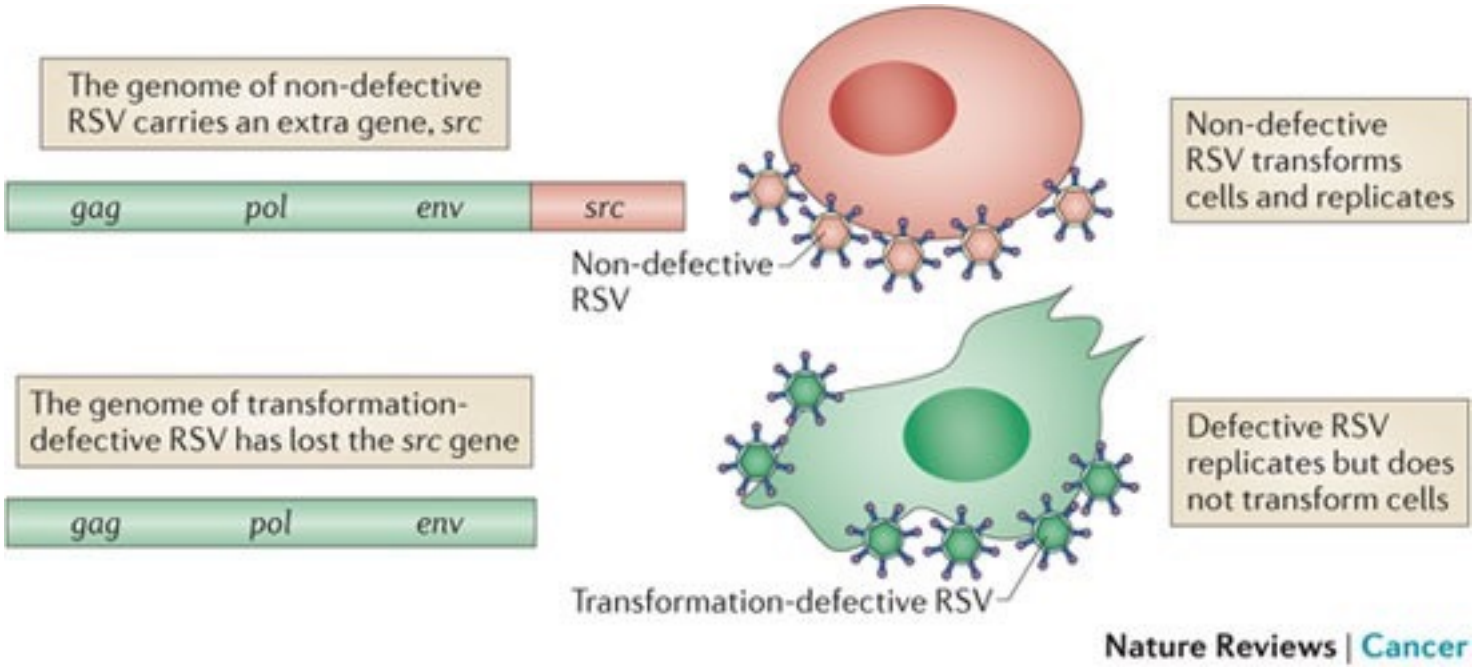
1) Very difficult to properly control away co-variates (Evian)
2) Think about effect size and timing
3) Be skeptical – even for RCTs, think about robustness, study populations, exclusion criteria



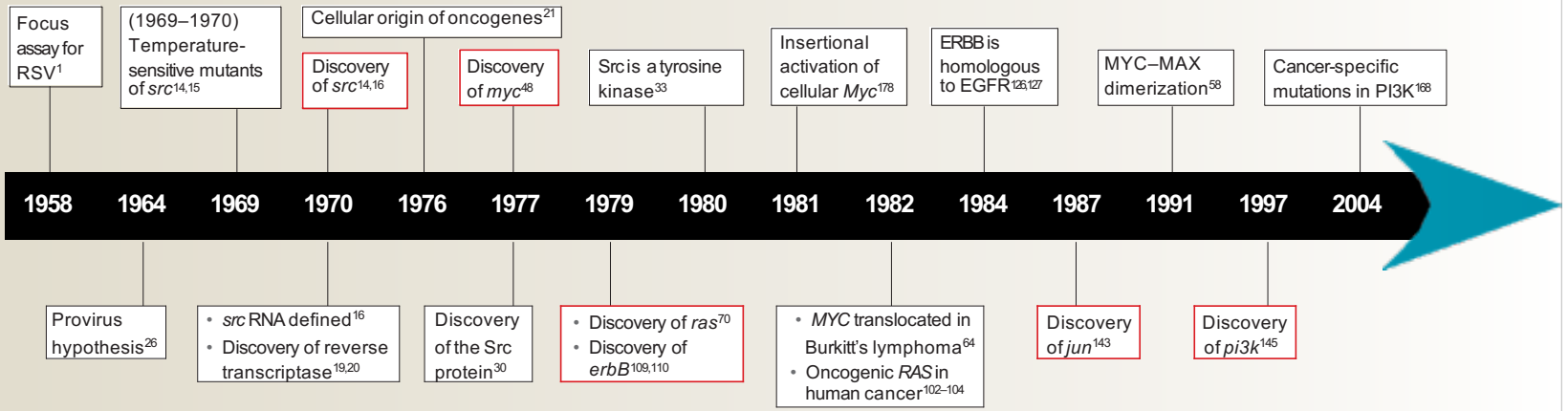
Holland-Frei Cancer Medicine, Ninth Edition. Edited by Robert C. Bast Jr., Carlo M. Croce, William N. Hait, Waun Ki Hong, Donald W. Kufe, Martine Piccart-Gebhart, Raphael E. Pollock, Ralph R. Weichselbaum, Hongyang Wang, and James F. Holland.

DOI: 10.1002/9781119000822.hfcm007

V-Src: The first oncogene



Timeline | **Retroviral oncogenes: 50 years of discovery**



The boxes outlined in black refer to discoveries that have shaped the research on oncogenic retroviruses. The boxes outlined in red mark the years in which important oncogenes were identified. EGFR, epidermal growth factor receptor; RSV, Rous sarcoma virus.

From proto-oncogene to oncogene: Recurrent RAS mutations

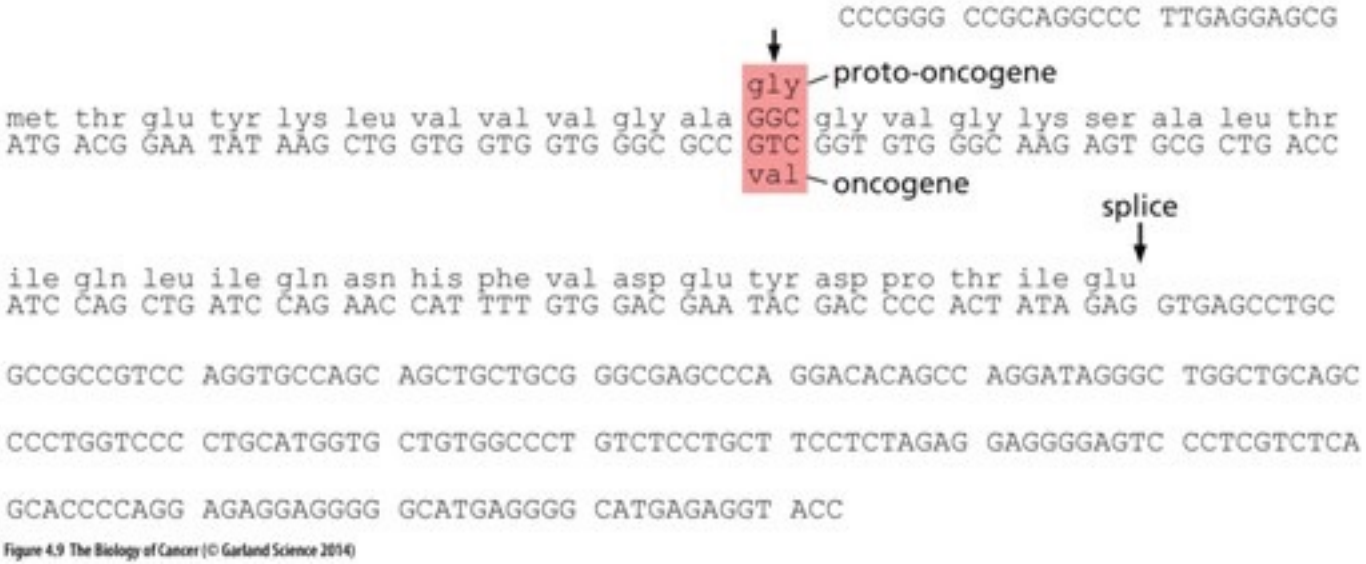


Figure 4.9 The Biology of Cancer (© Garland Science 2014)

From proto-oncogene to oncogene: MYCN overexpression

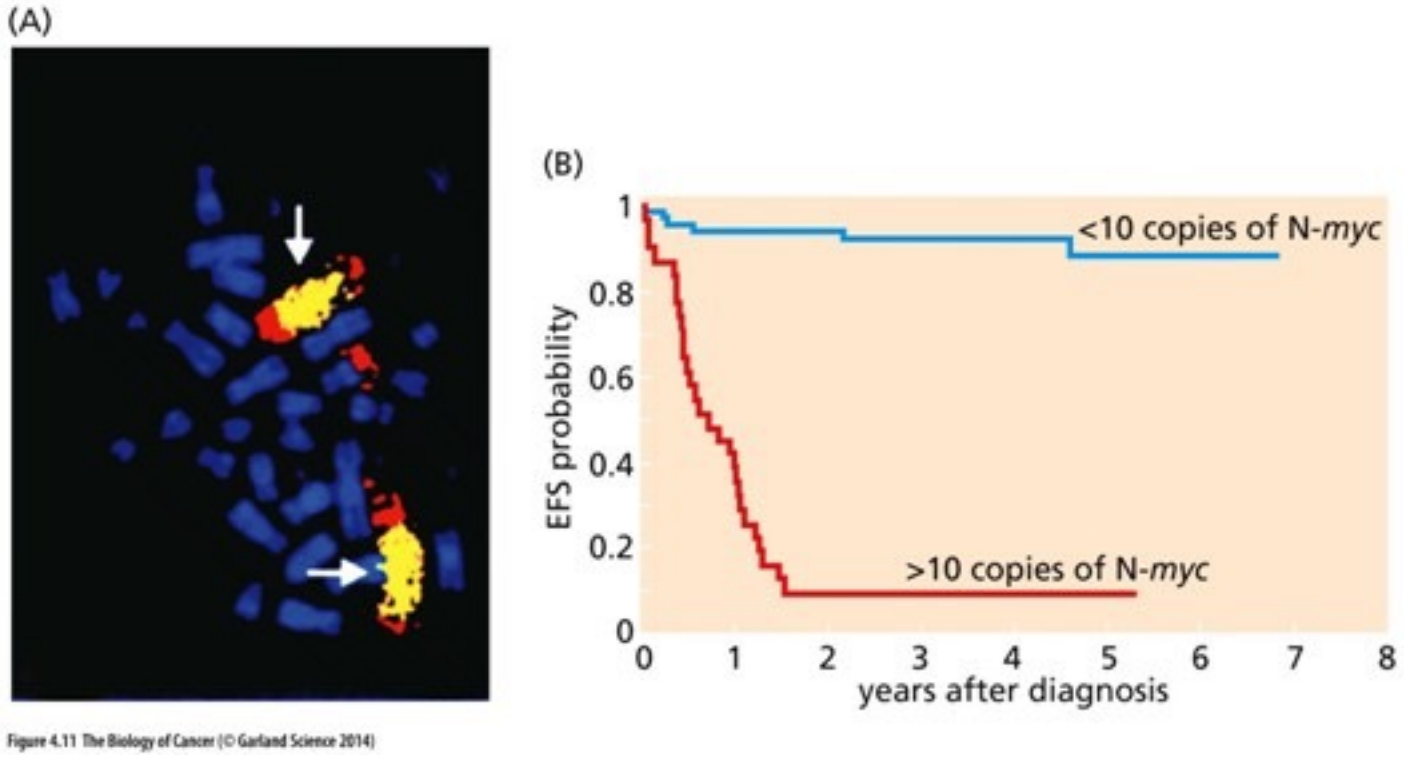
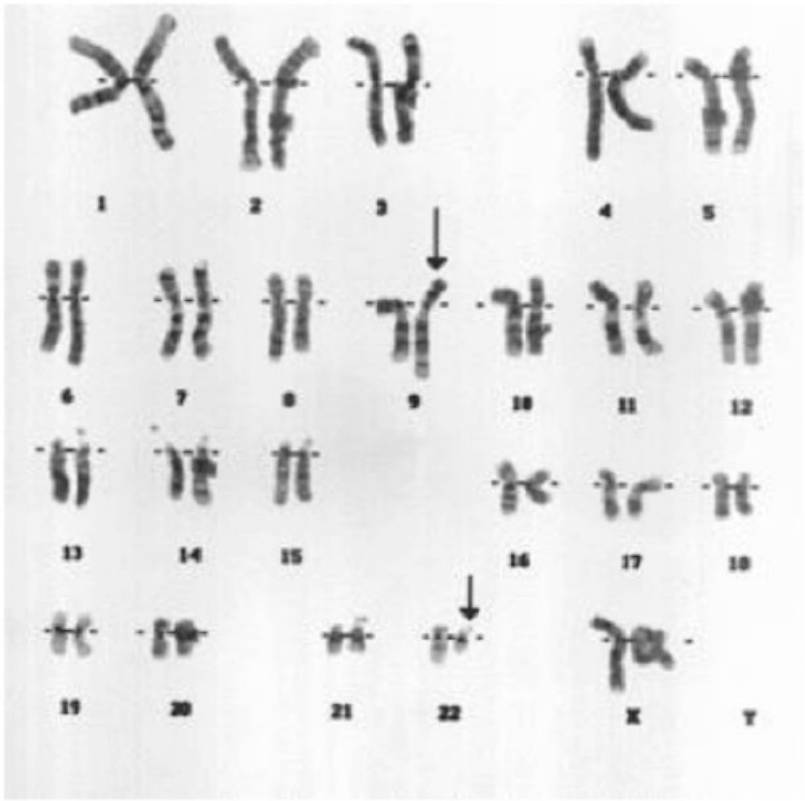
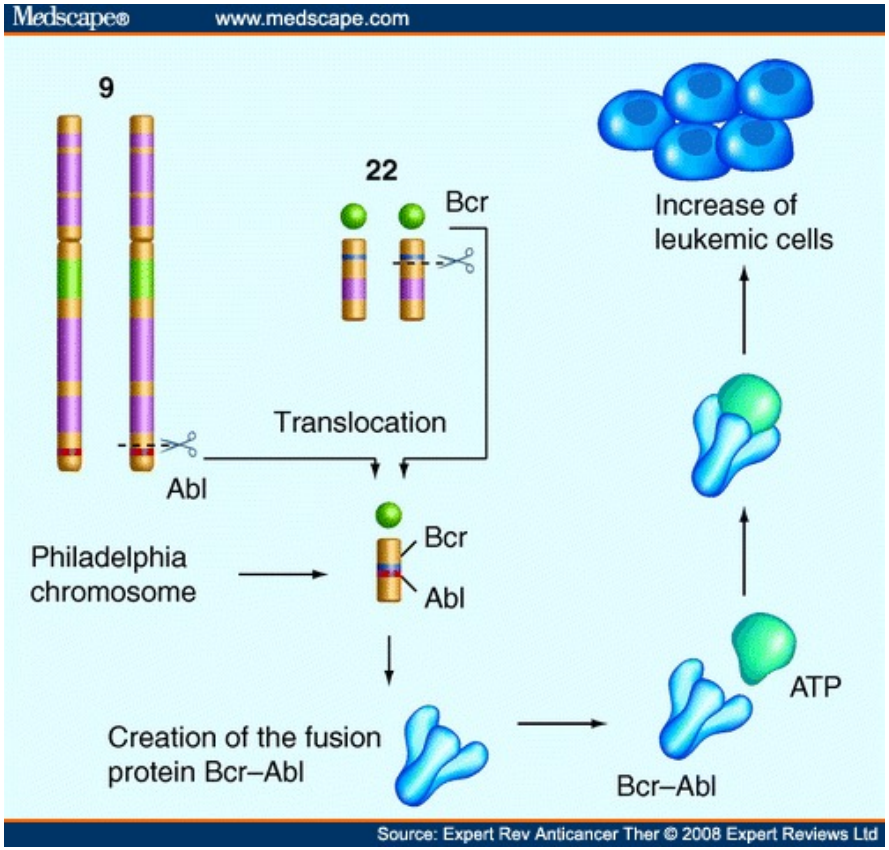


Figure 4.11 The Biology of Cancer (© Garland Science 2014)

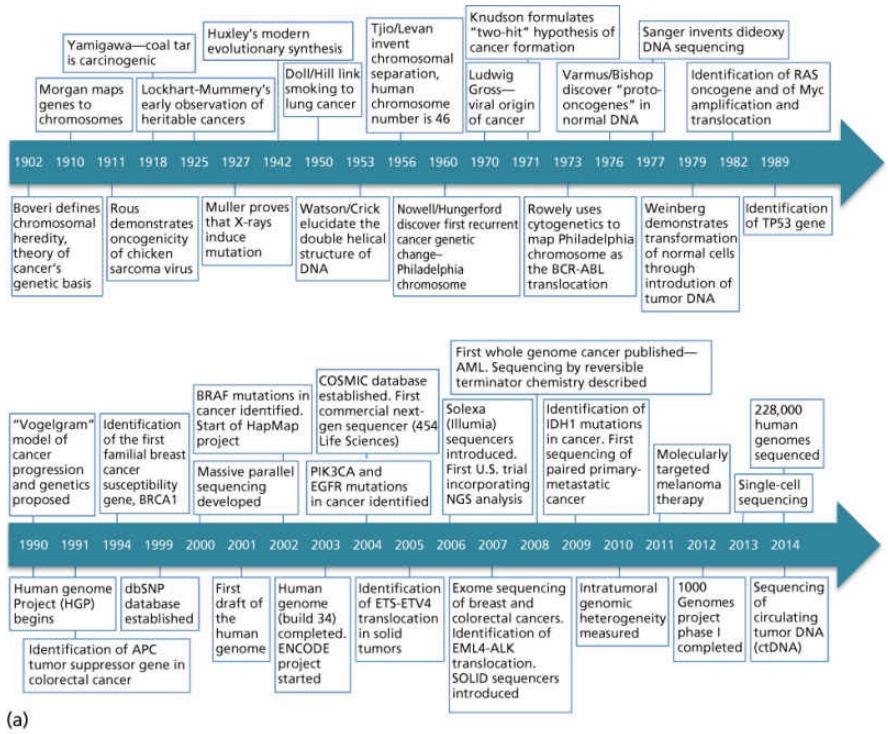
From proto-oncogene to oncogene: Chromosomal rearrangements (BCR-ABL)



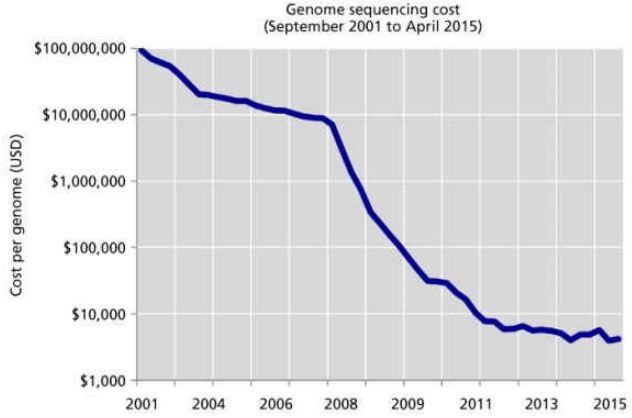
The abnormality seen by Nowell & Hungerford on chromosome 22, Now known as the Philadelphia Chromosome.



The genomics revolution in cancer biology

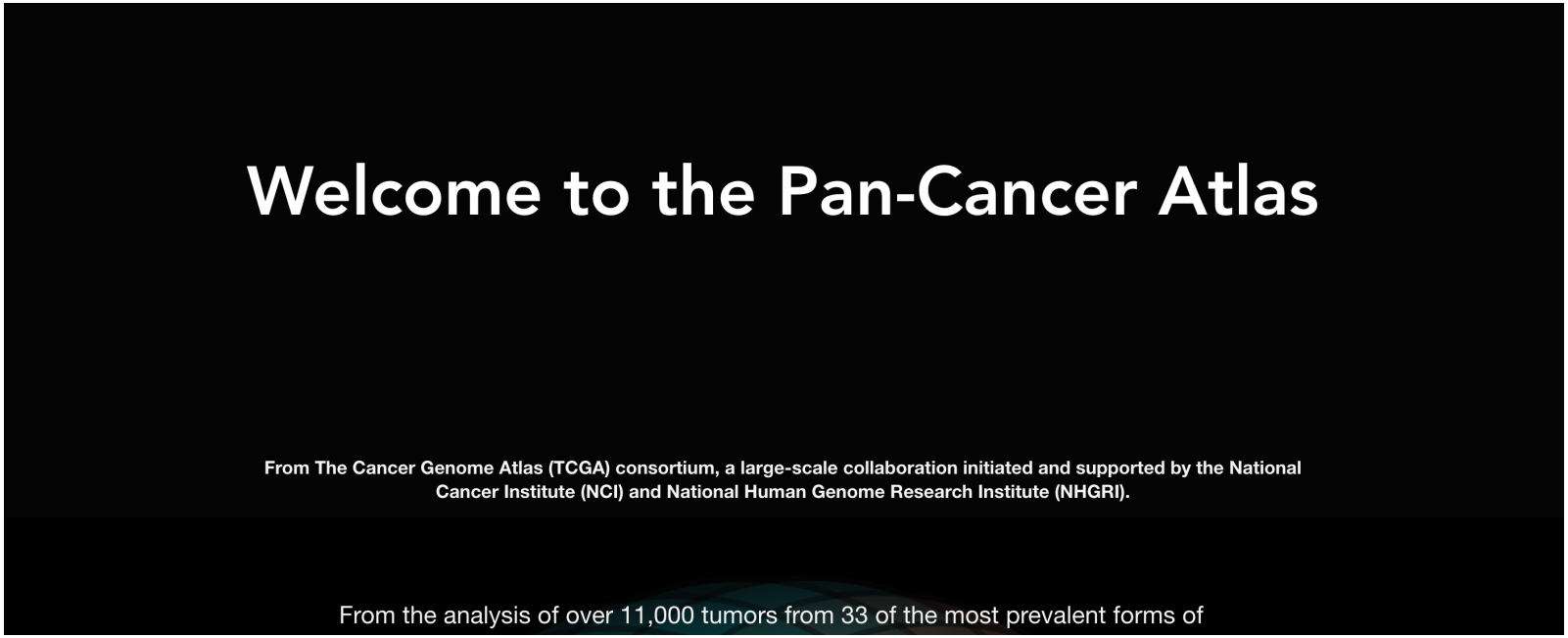


(a)



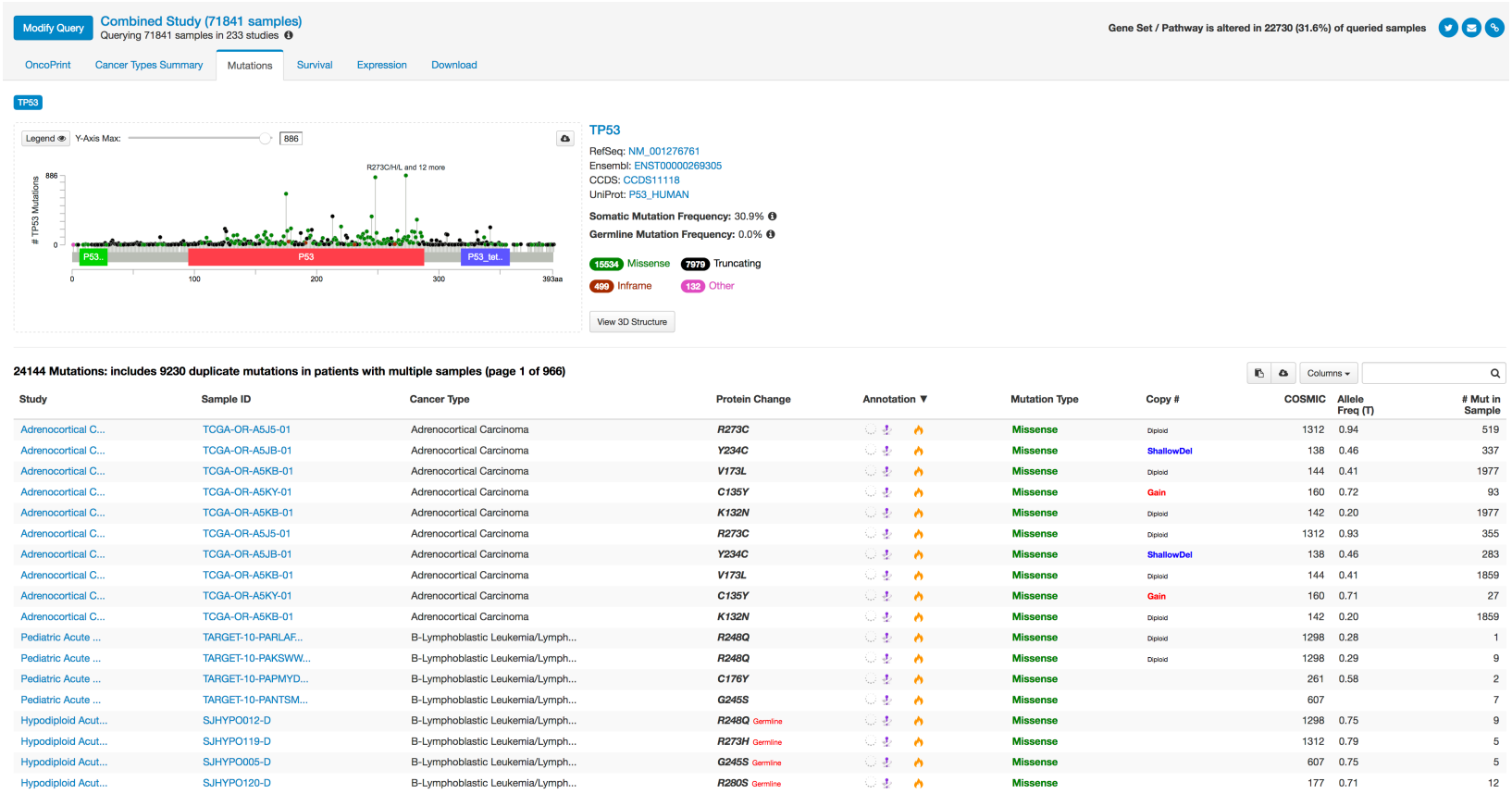
(b)

The genomics revolution in cancer biology



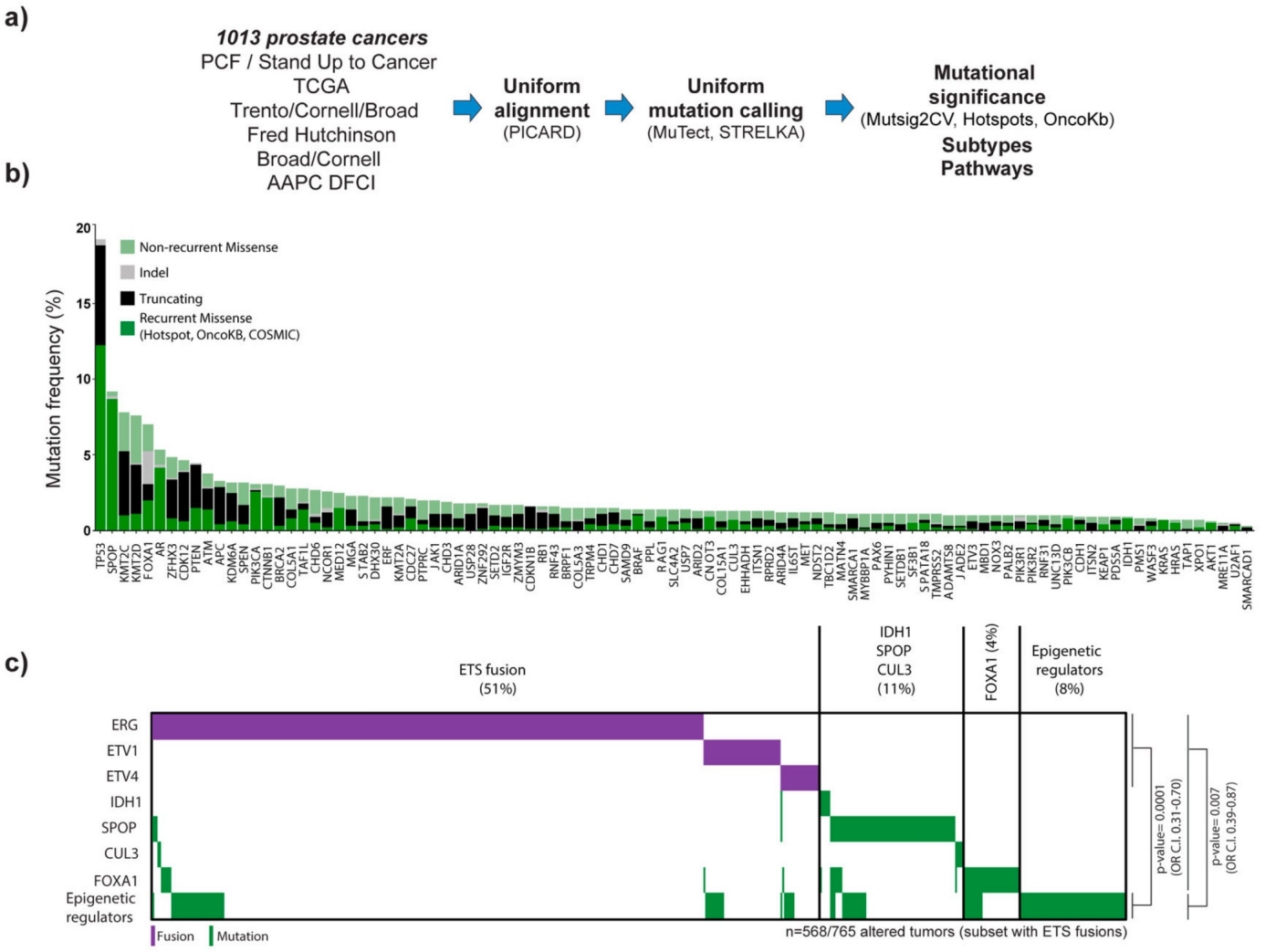
https://www.cell.com/pb-assets/consortium/pancanceratlas/pancani3/index.html

The genomics revolution in cancer biology



Study	Sample ID	Cancer Type	Protein Change	Annotation ▼	Mutation Type	Copy #	COSMIC	Allele Freq (T)	# Mut in Sample
Adrenocortical C...	TCGA-OR-A5J5-01	Adrenocortical Carcinoma	*R273C*		Missense	Diploid	1312	0.94	519
Adrenocortical C...	TCGA-OR-A5JB-01	Adrenocortical Carcinoma	*Y234C*		Missense	ShallowDel	138	0.46	337
Adrenocortical C...	TCGA-OR-A5KB-01	Adrenocortical Carcinoma	*V173L*		Missense	Diploid	144	0.41	1977
Adrenocortical C...	TCGA-OR-A5KY-01	Adrenocortical Carcinoma	*C135Y*		Missense	Gain	160	0.72	93
Adrenocortical C...	TCGA-OR-A5KB-01	Adrenocortical Carcinoma	*K132N*		Missense	Diploid	142	0.20	1977
Adrenocortical C...	TCGA-OR-A5J5-01	Adrenocortical Carcinoma	*R273C*		Missense	Diploid	1312	0.93	355
Adrenocortical C...	TCGA-OR-A5JB-01	Adrenocortical Carcinoma	*Y234C*		Missense	ShallowDel	138	0.46	283
Adrenocortical C...	TCGA-OR-A5KB-01	Adrenocortical Carcinoma	*V173L*		Missense	Diploid	144	0.41	1859
Adrenocortical C...	TCGA-OR-A5KY-01	Adrenocortical Carcinoma	*C135Y*		Missense	Gain	160	0.71	27
Adrenocortical C...	TCGA-OR-A5KB-01	Adrenocortical Carcinoma	*K132N*		Missense	Diploid	142	0.20	1859
Pediatric Acute ...	TARGET-10-PARLAF...	B-Lymphoblastic Leukemia/Lymph...	*R248Q*		Missense	Diploid	1298	0.28	1
Pediatric Acute ...	TARGET-10-PAKSWW...	B-Lymphoblastic Leukemia/Lymph...	*R248Q*		Missense	Diploid	1298	0.29	9
Pediatric Acute ...	TARGET-10-PAPMYD...	B-Lymphoblastic Leukemia/Lymph...	*C176Y*		Missense		261	0.58	2
Pediatric Acute ...	TARGET-10-PANTSM...	B-Lymphoblastic Leukemia/Lymph...	*G245S*		Missense		607		7
Hypodiploid Acut...	SJHYPO012-D	B-Lymphoblastic Leukemia/Lymph...	*R248Q* Germline		Missense		1298	0.75	9
Hypodiploid Acut...	SJHYPO119-D	B-Lymphoblastic Leukemia/Lymph...	*R273H* Germline		Missense		1312	0.79	5
Hypodiploid Acut...	SJHYPO005-D	B-Lymphoblastic Leukemia/Lymph...	*G245S* Germline		Missense		607	0.75	5
Hypodiploid Acut...	SJHYPO120-D	B-Lymphoblastic Leukemia/Lymph...	*R280S* Germline		Missense		177	0.71	12

A lot of alterations in cancer: What defines an oncogene?

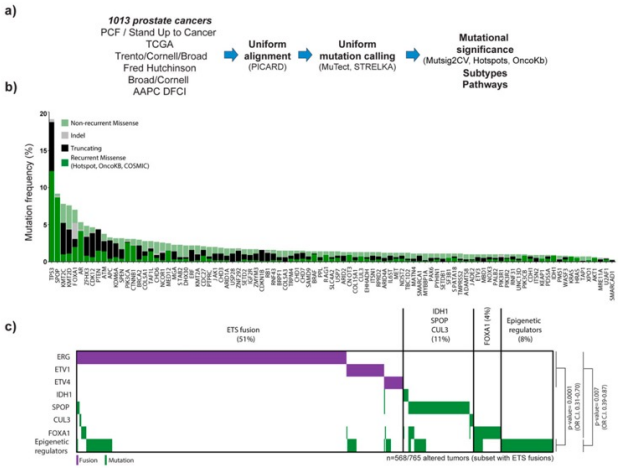


Armenia et al. *Nature Genetics* 2018

Terminology

Types of Alterations (tumor intrinsic..):

Mutations (promoters, introns too..)
Insertions/Deletions
Copy Number Changes
Epigenetic alterations (methylation)



Classifying alterations:
Driver mutations (BCR-ABL in CML not ALL, EGFRL858R, KRASG12V…)
Passenger mutations
In-between – the long tail of mutations in cancer

Tumor Suppressor (p53 loss – is this a “driver”?)
Oncogene
Genetic Heterogeneity

Microenvironment/Immune Detection

Back to the beginning: what defines an oncogene?

Classic transformation assay

Transforming genes of human bladder and lung carcinoma cell lines are homologous to the *ras* genes of Harvey and Kirsten sarcoma viruses

(human tumors/transfection/retroviruses)

CHANNING J. DER, THEODORE G. KRONTIRIS, AND GEOFFREY M. COOPER

Sidney Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

Communicated by Baruj Benacerraf, March 22, 1982

ABSTRACT Blot hybridization analysis indicated that NIH 3T3 mouse cells transformed by high molecular weight DNAs of a human bladder and a human lung carcinoma cell line contained new sequences homologous, respectively, to the transforming genes of Harvey (*ras*^{H}) and Kirsten (*ras*^{K}) sarcoma viruses. The unique *ras* sequences were present in multiple independent NIH cell lines transformed in both primary and secondary transfection assays and corresponded to *ras* sequences normally present in human DNAs. The *ras* gene product was expressed in NIH cells transformed by bladder carcinoma DNAs and in the human bladder carcinoma cell lines at levels 2- to 4-fold greater than the level observed in nontransformed NIH 3T3 cells. These results indicate that the transforming genes of these human tumor cell lines are the cellular homologs of two retroviral transforming genes.

Back to the beginning: what defines an oncogene?

1. Recurrent mutations in cancer and not normal tissue
   - what about tumor suppressors
   - what if the mutation does show up in normal tissues (BRAFV600E)

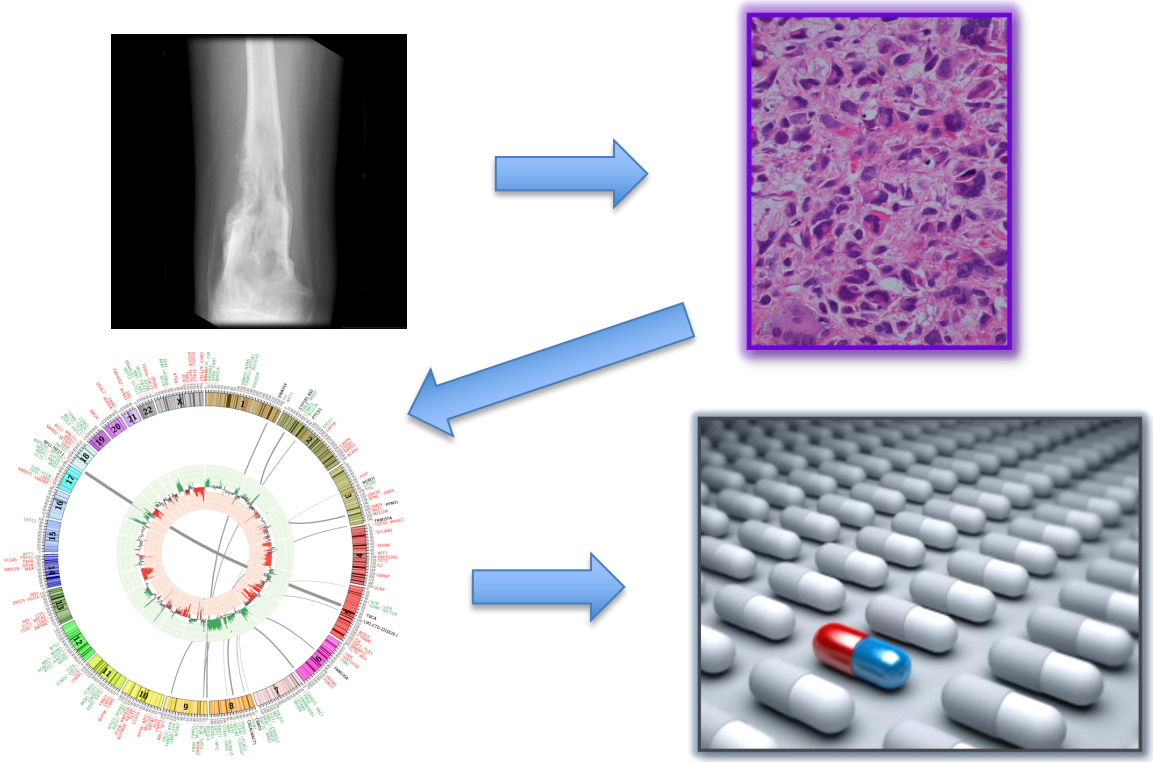
2. Cell Transformation Assays: Normal cells -> cancer
   - 3T3 (mouse!) or other colony forming assays
   - Ba/F3 – IL-3 dependence

3. Tumors in mice?
   - what if it can't? EWS-FLI1. lineage specificity
   - transgenic vs xenografts

4. Essential for the growth of cancer cell?
   - essential genes
   - concept of driver vs passenger.

5. On-target clinical resistance to targeted therapies

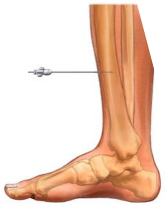
If this is the goal of precision medicine…



The reality is more complicated and disease-dependent.

How we treat pediatric sarcoma (bone tumors)

NOW



Biopsy

Ewing's Sarcoma

Osteosarcoma

Rhabdomyosarcoma

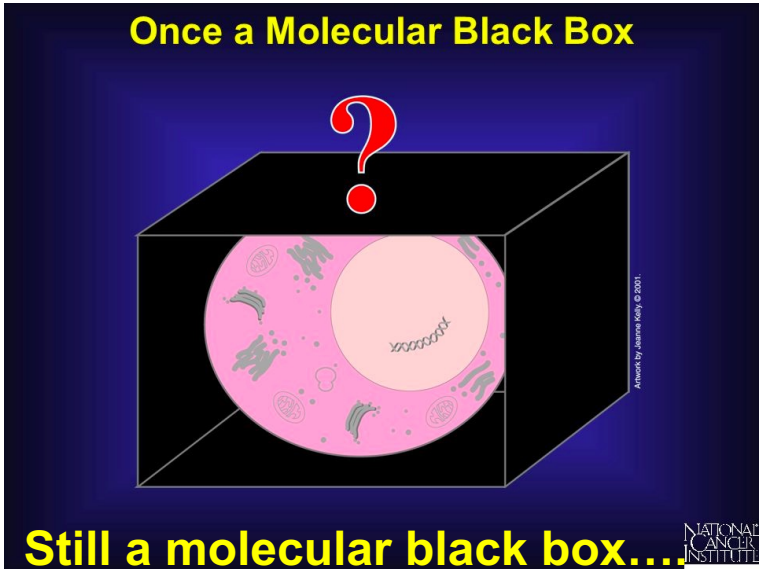
Chemotherapy

Radiation/Surgery

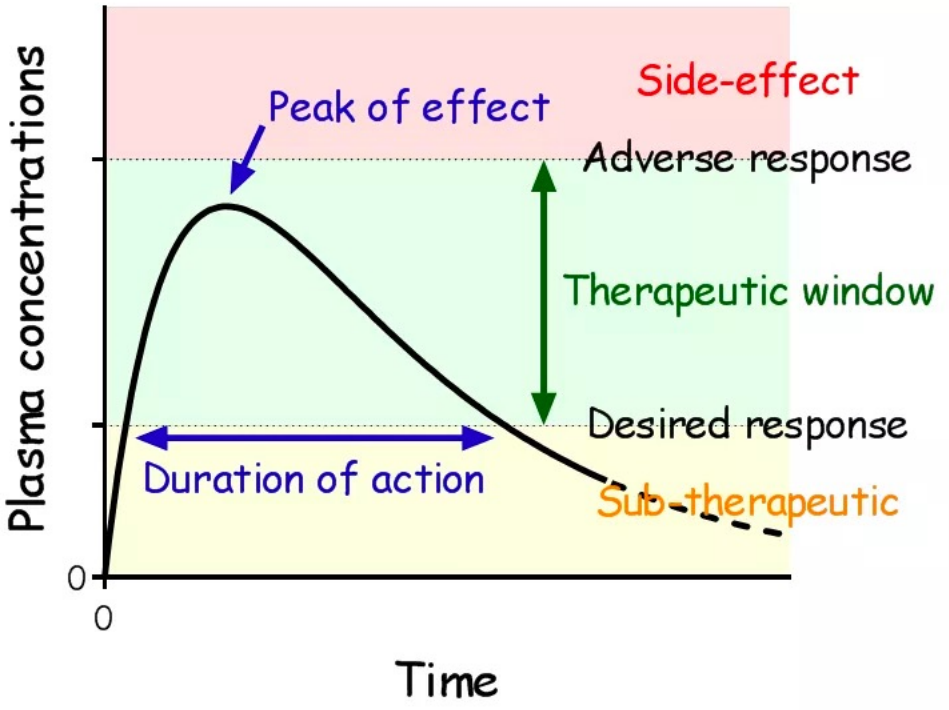
The concept of a therapeutic window

How does doxorubicin work? DNA damage
How does vincristine work? MT inhibitor
Bortezomib in myeloma?

Why do these drugs show any selectivity for cancer cells?
Why is their histologic variation in response and why do they stop working (ie resistance mechanisms)?



What is the biologic basis of a therapeutic window?



Ex: Vemurafenib in BRAF mutant melanoma

But what about DNA-damaging agents??

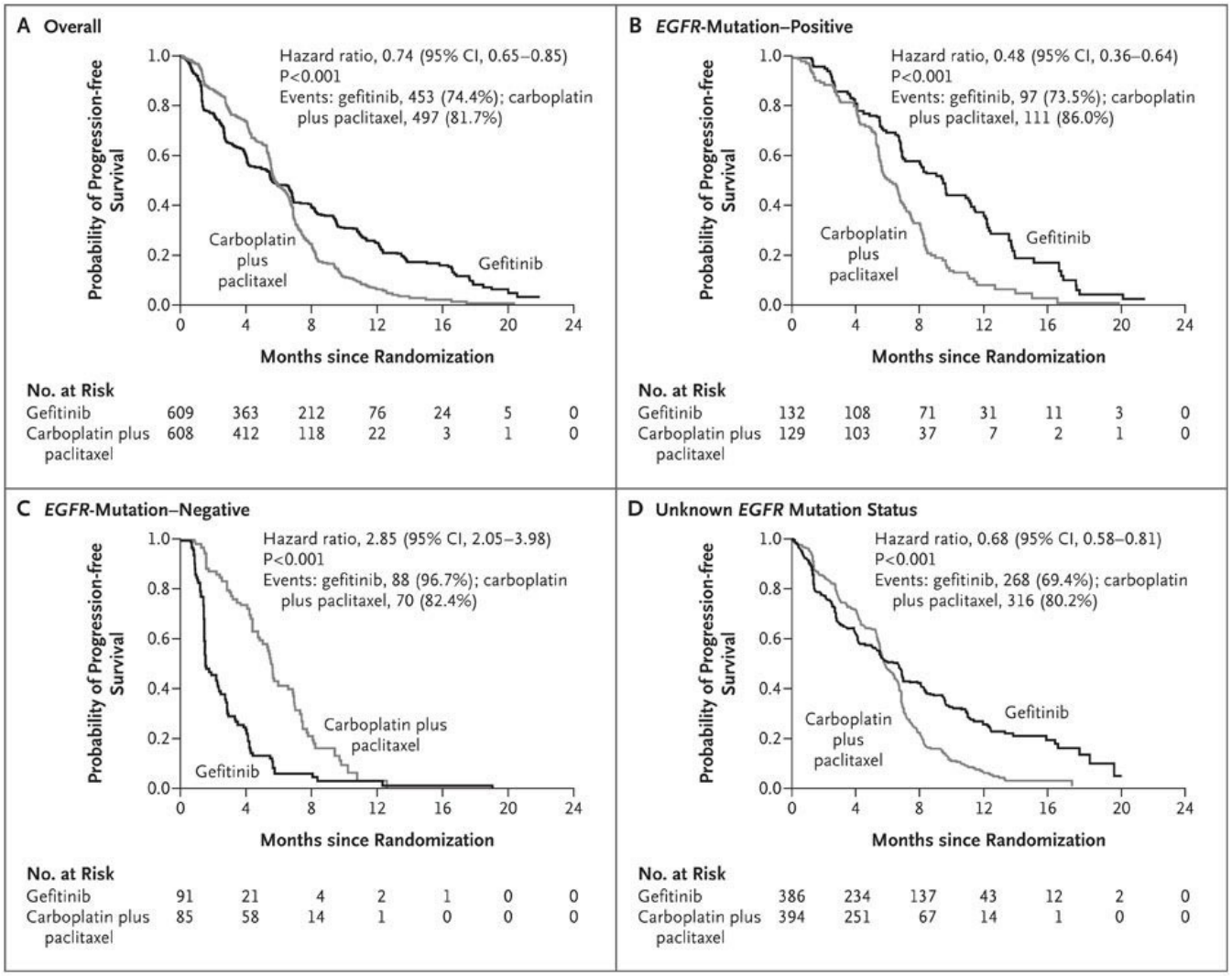
We need to identify driver oncogenes and design drugs to target them (maybe?)

Kinase mutations/Fusions:
EGFR in lung cancer
BCR-ABL in CML
BRAFV600E in melanoma
Crizotinib in ALK fusion cancers
RAS inhibitors

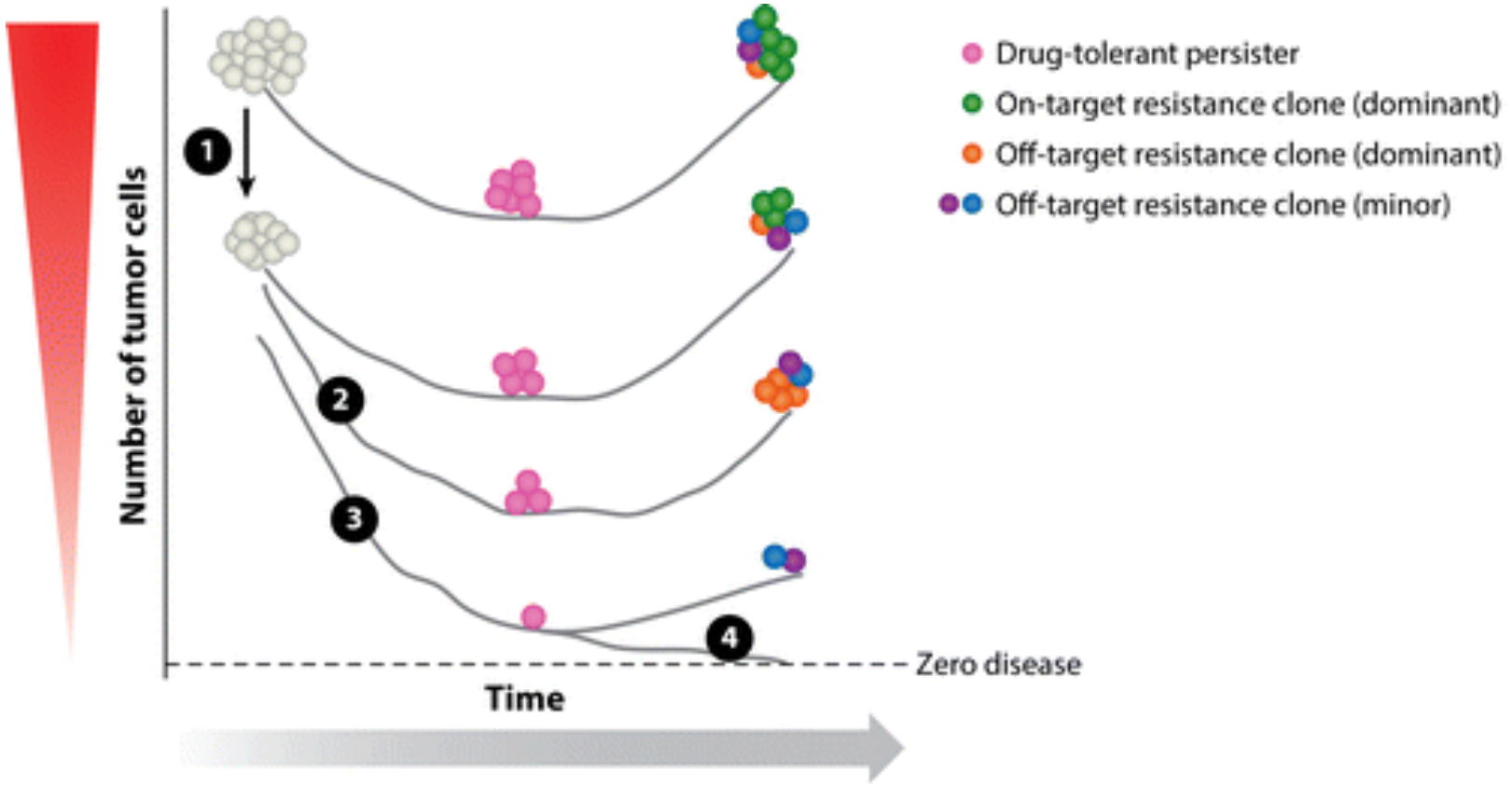
Antibody-based Therapies:
Herceptin in ER-positive breast CA
Brenutximab in CD30+ ALCL
Cetuximab in Head and Neck

I won't mention immunotherapies (CAR-T, PD1)

EGFR in lung cancer

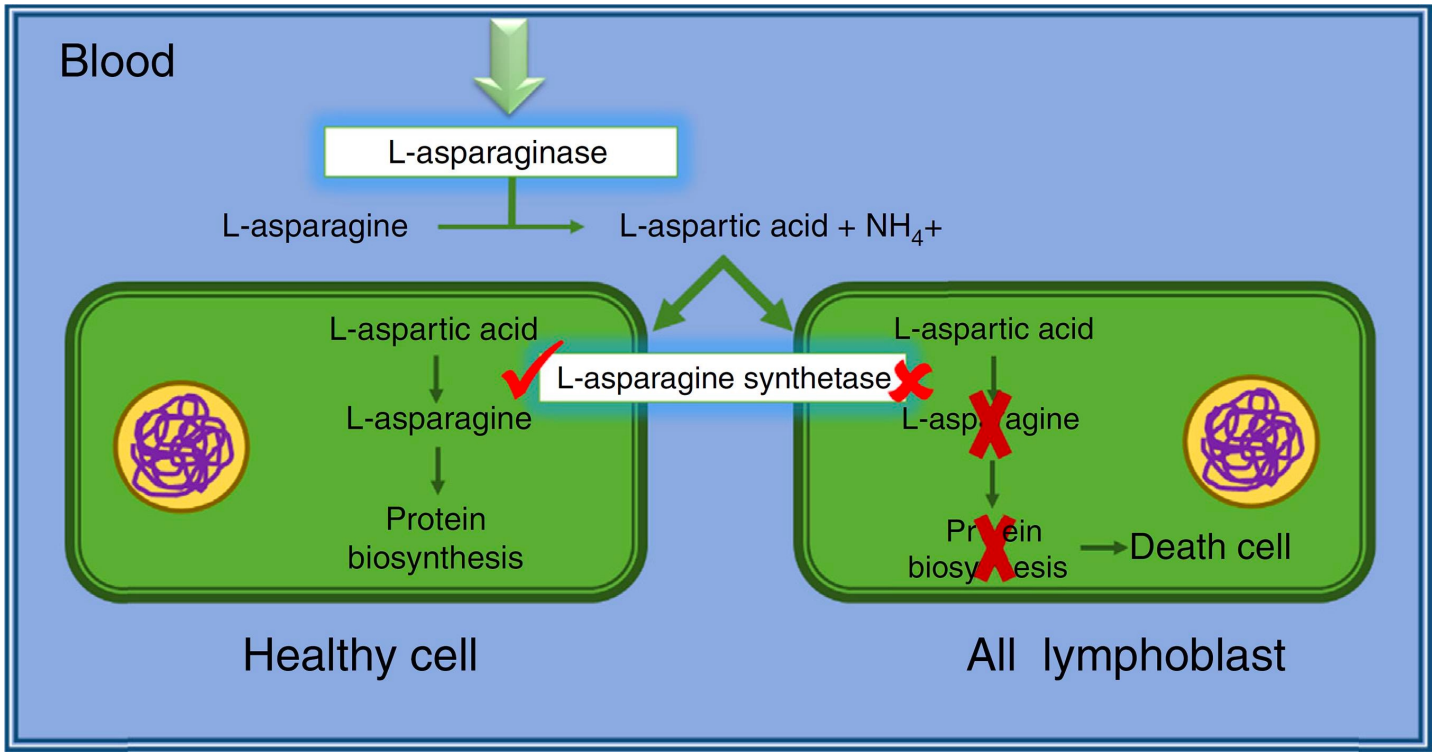


Mok et al, NEJM 2009



Tulpule A, Bivona TG. 2020.
Annu. Rev. Cancer Biol. 4:279–97

What is a cancer dependency?



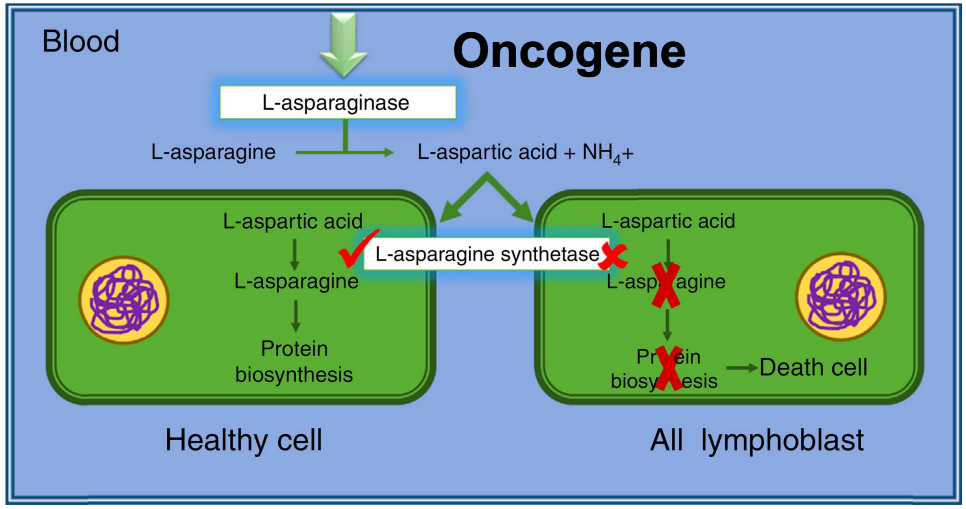
Asparaginase in ALL

If there is a classic activating KRAS mutation in a tumor, is that tumor dependent on KRAS?

What is a cancer dependency?

My definition (too long!....):

Cancers of a certain <u>class</u> or <u>genotype</u>, by virtue of their specific biology, display selective dependence on a gene/pathway or selective lethality in the setting of a drug, when compared with "normal" cells.

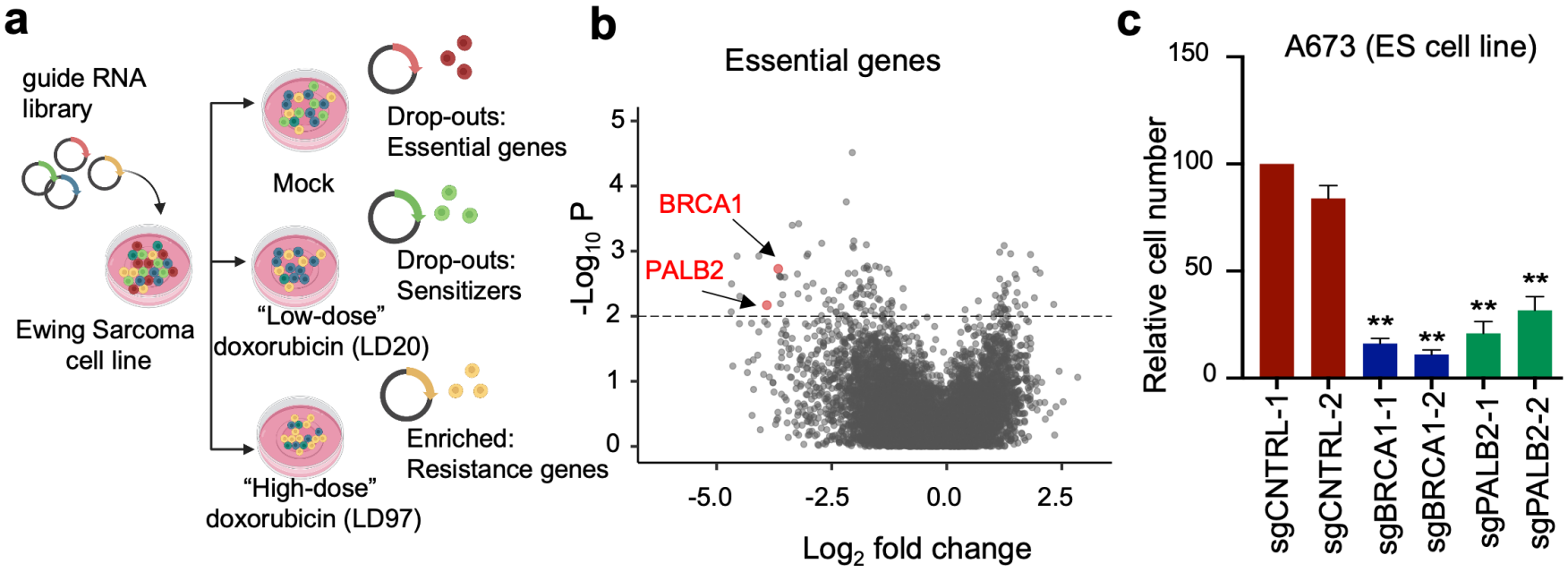


Asparaginase vs bleach/ribosomal proteins

How to (critically) read a paper

1. Understand the scientific question. If not in your field, ideally read the cited papers AND search the literature yourself. Do not just accept the authors framing.
2. For each figure panel, first interpret the data yourself. Think about the results in the context/limitations of the technique and approaches (e.g., CRISPR screening). Then, assess the authors conclusions and see if you agree and/or other explanations are warranted.
3. A question to always ask: what is the least interesting explanation for this result? What controls are missing that would undermine the authors conclusions?
4. When presenting papers in journal club, provide your interpretation first by discussing strengths of a figure and limitations - do NOT just read the authors interpretation!
5. Conclusions – this is the hardest part. Assess the totality of the evidence.

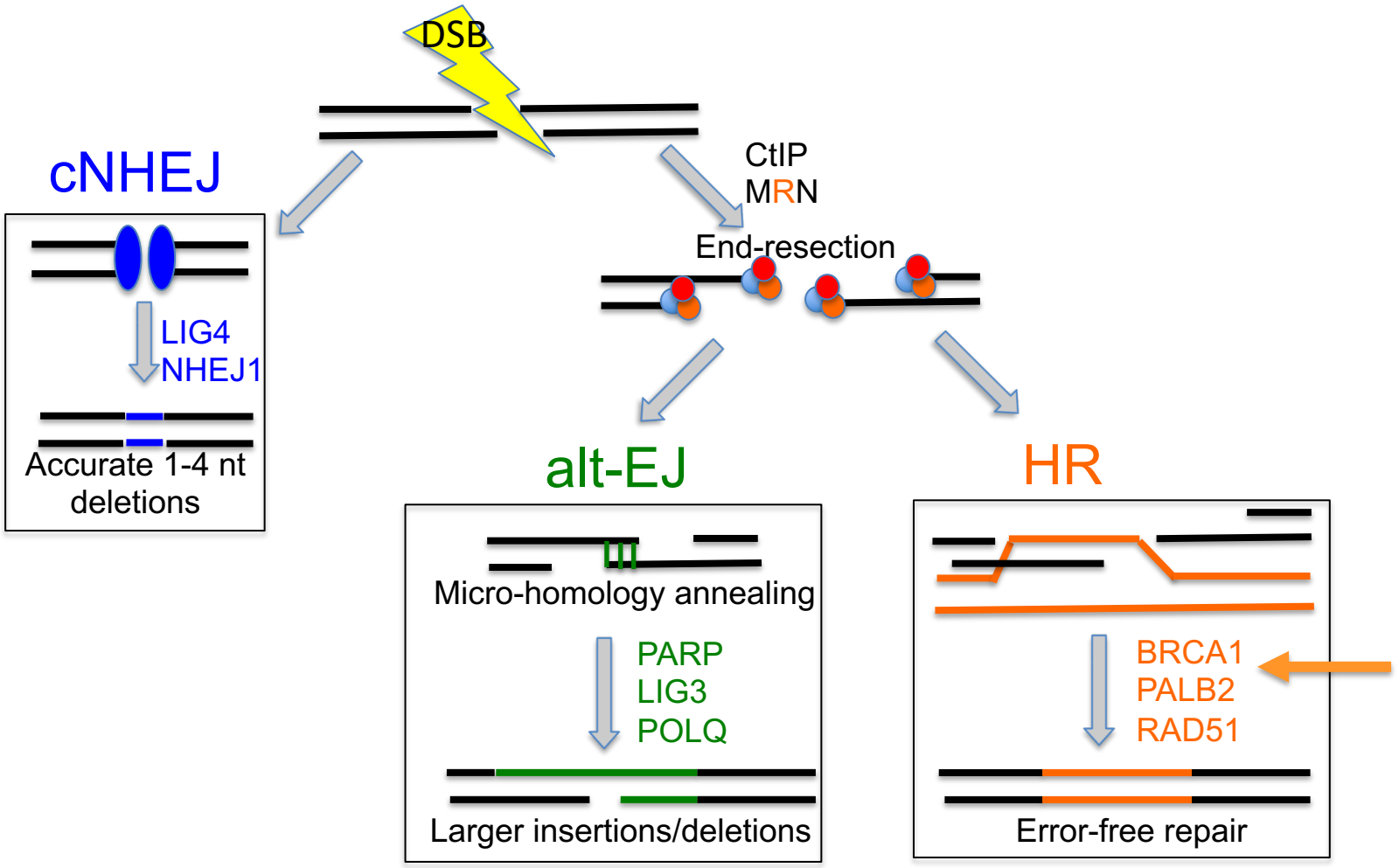
Figure 1



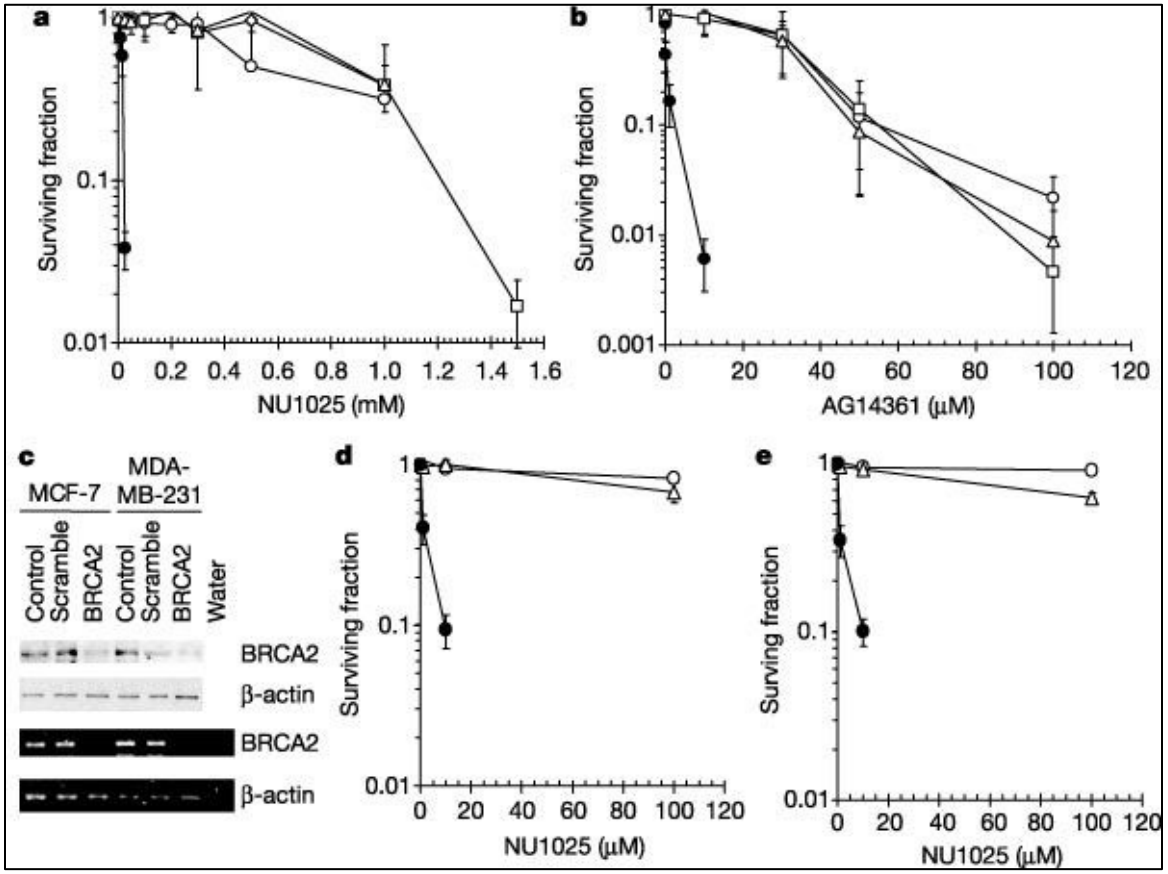
Ewing sarcoma cells are dependent on specific HR factors (BRCA1, PALB2) for survival. Are they?

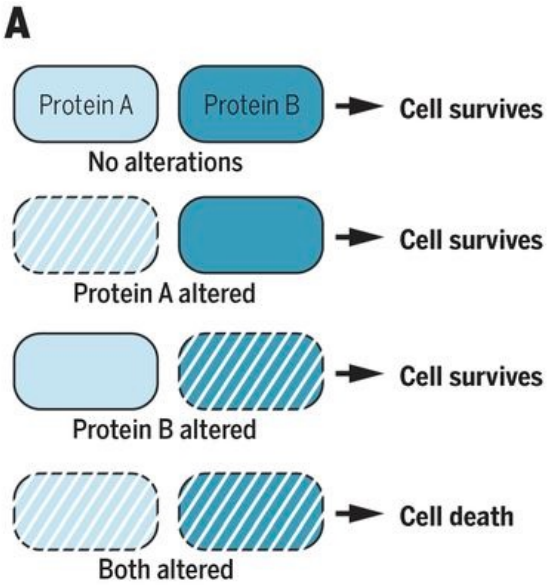
1. Validity of actual finding
2. Robustness
3. Reproducibility/relevant biology

Cancer dependency/synthetic lethality
Example 1: Hereditary Breast Cancer and BRCA1



Synthetic Lethality: BRCA1 loss and PARP

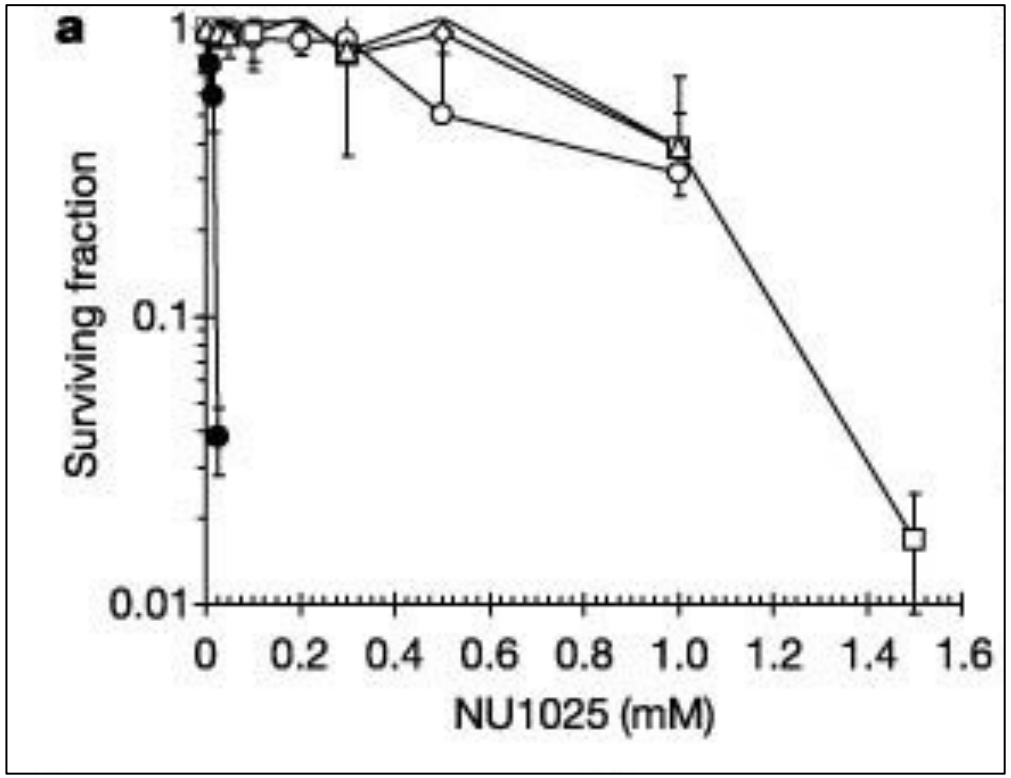




Bryant et al., 2005

So this brings up a key question…

How do you test whether a cancer dependency is real?



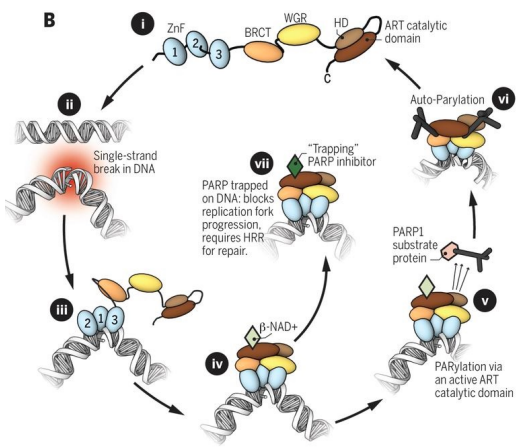
These data suggest BRCA2 mutant cancer cells are synthetic lethal with PARP inhibitors/dependent on PARP. Are they?

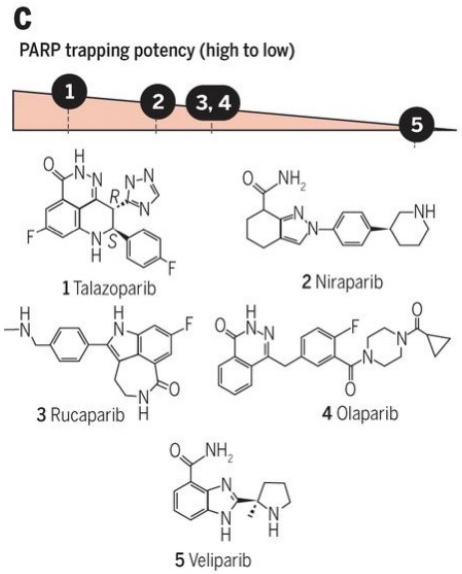
Testing a cancer dependency…

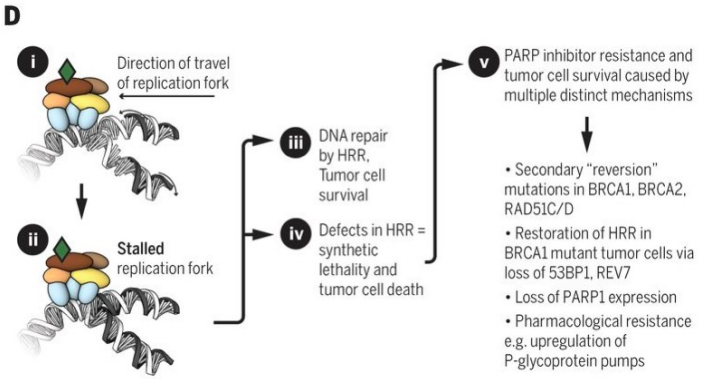
- Rescue the phenotype (add back BRCA1)
- Orthogonal approaches (genetics)
- Cell Lines (how generalizable?)
- Xenografts, PDXs – better gauge of therapeutic window

- Clinical Trials (not always feasible)
- Resistance Mutations

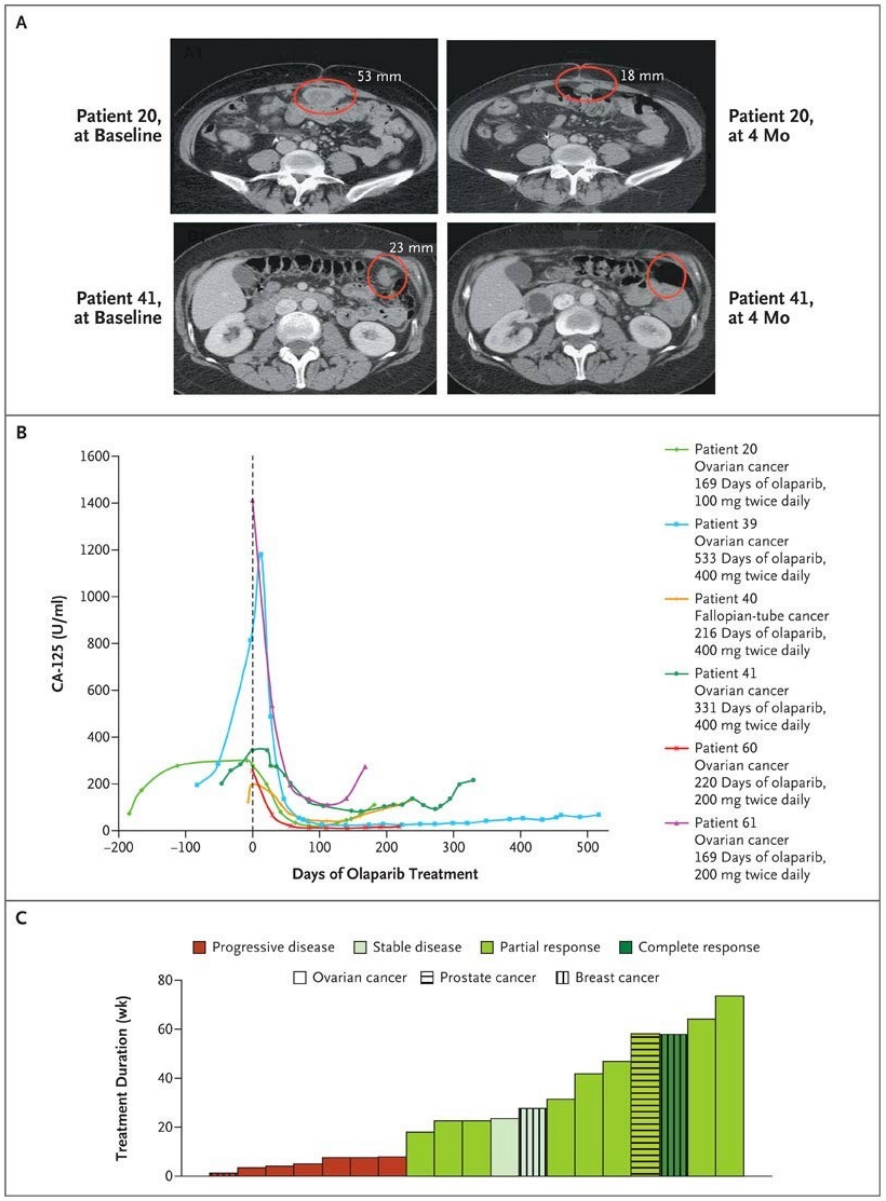
Mechanism of PARPi/BRCA synthetic lethality??







Testing cell line findings in orthogonal ways (genetic PARP kd)
Strong cell line-based evidence that HR deficient cells are more sensitive to PARPi
Many mechanisms = we don't fully understand….

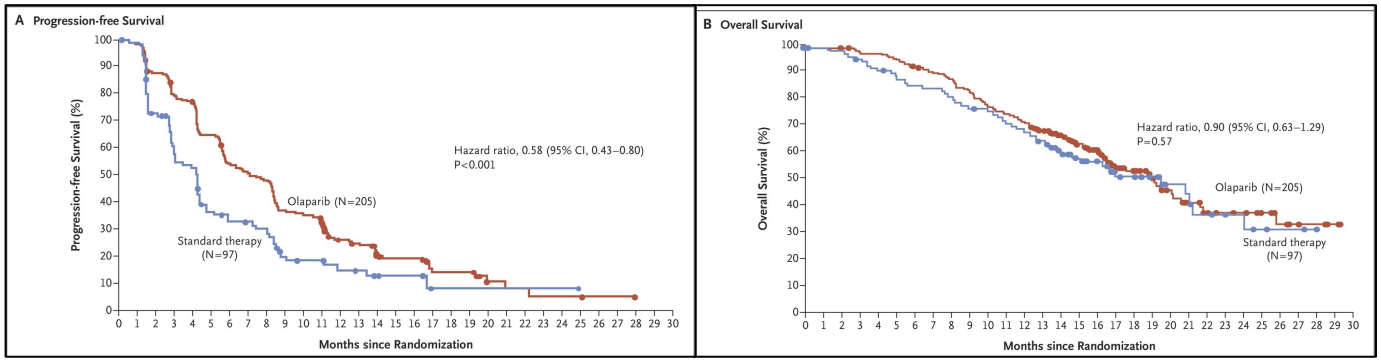


NEJM 2009, Fong et al.

First clinical demonstration of tumor response in BRCA mutant breast, ovarian, prostate CA

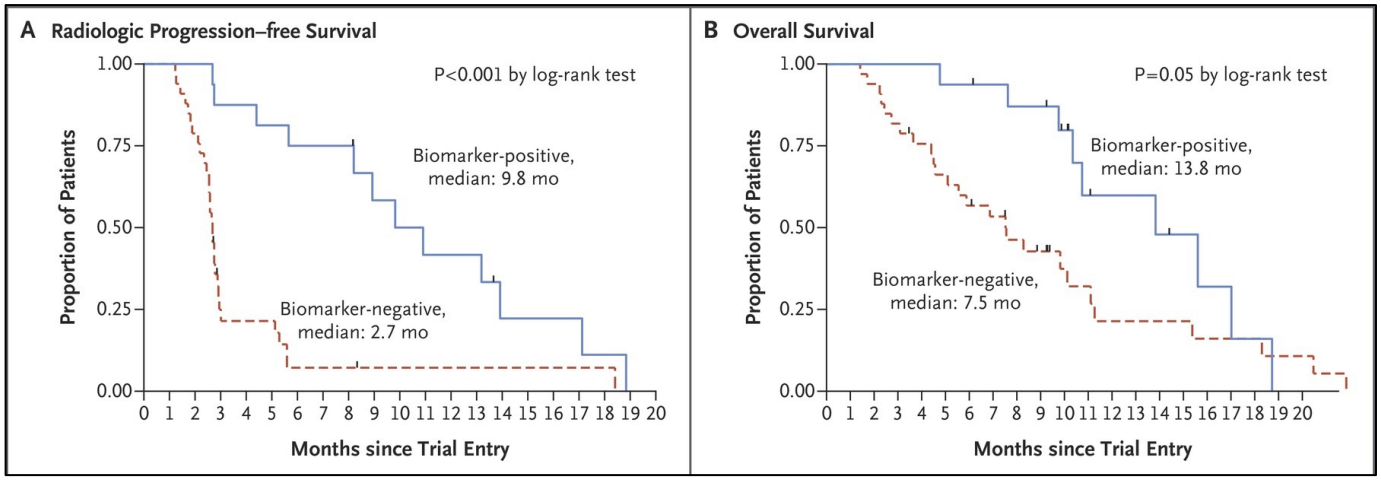
Clinical Outcomes

Breast CA



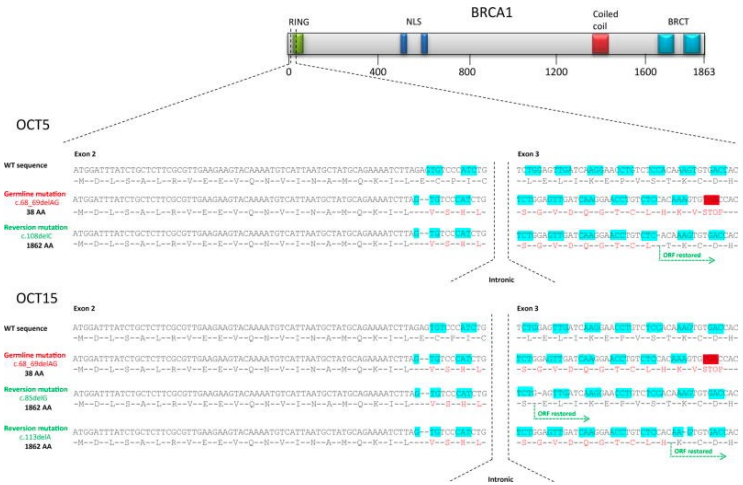
Robson et al, NEJM 2017

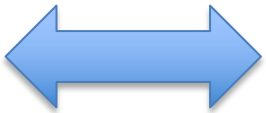
Prostate CA

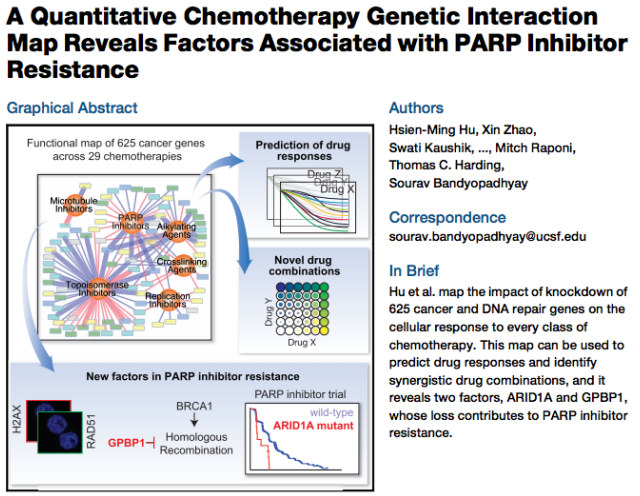


Mateo et al, NEJM 2014

Resistance as proof of dependency



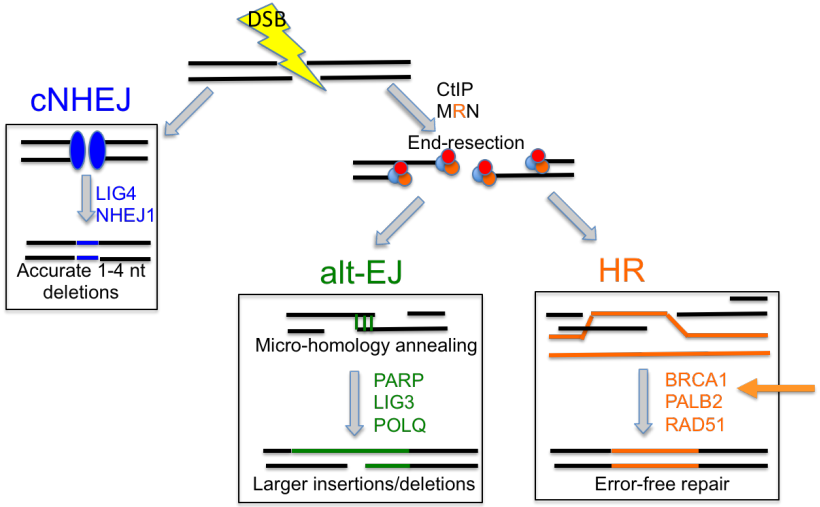




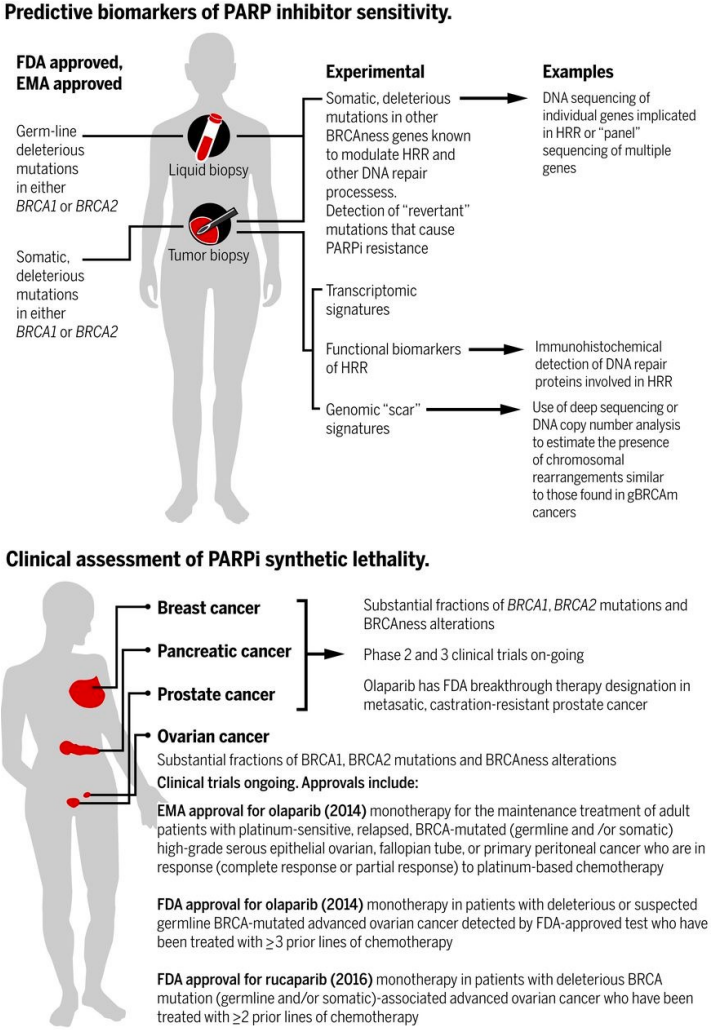
BRCA mutant patients develop reversion mutations after PARPi therapy

Race is on to identify mediators of PARPi resistance – can actually serve as clues to the real mechanism

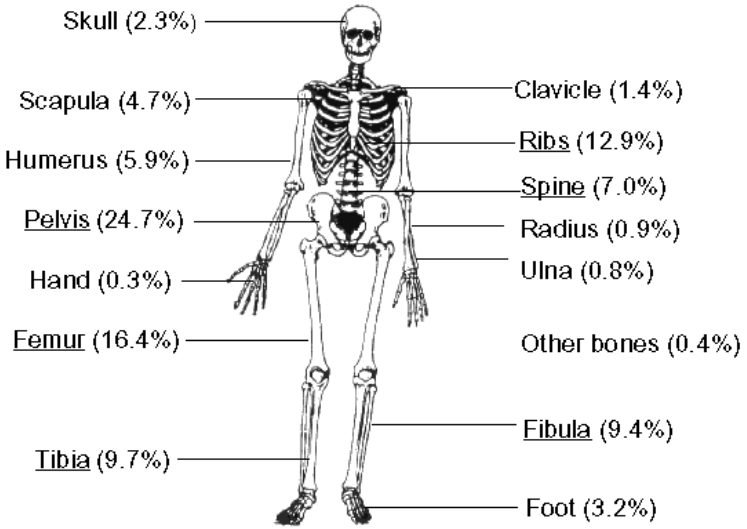
Can the PARPi/BRCAmut dependency be extended?



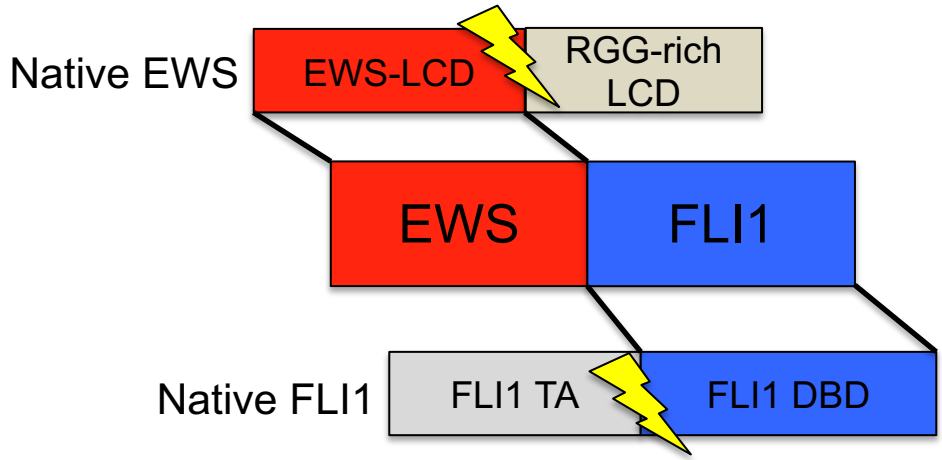
"Correlation + Plausibility does not equal causation"



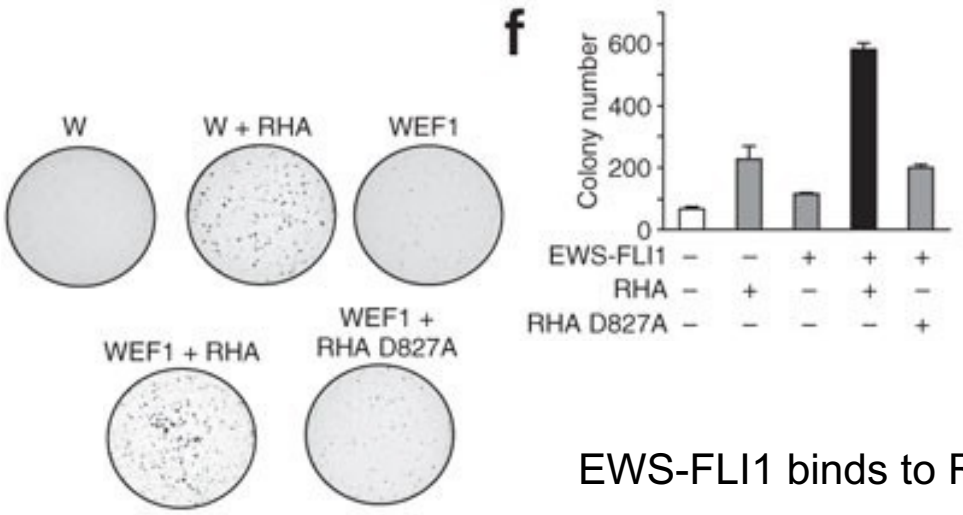
FET fusions in cancer: Ewing sarcoma



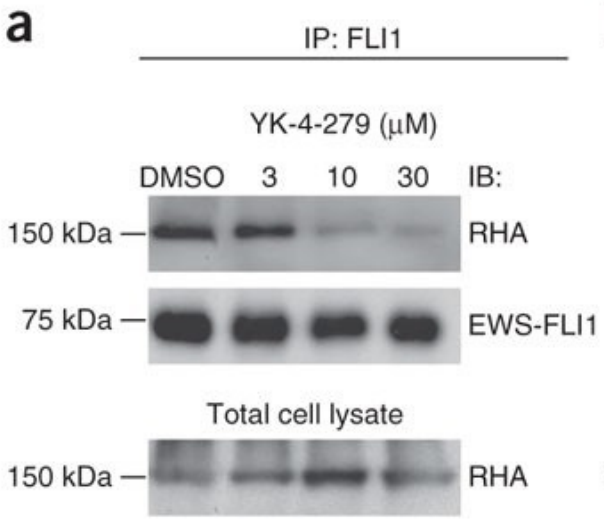
- Pediatric bone tumor
- EWS-FLI1 sole genetic driver
- "Melts like snow" (DNA repair defect?)



The EWS-FLI1 inhibitor

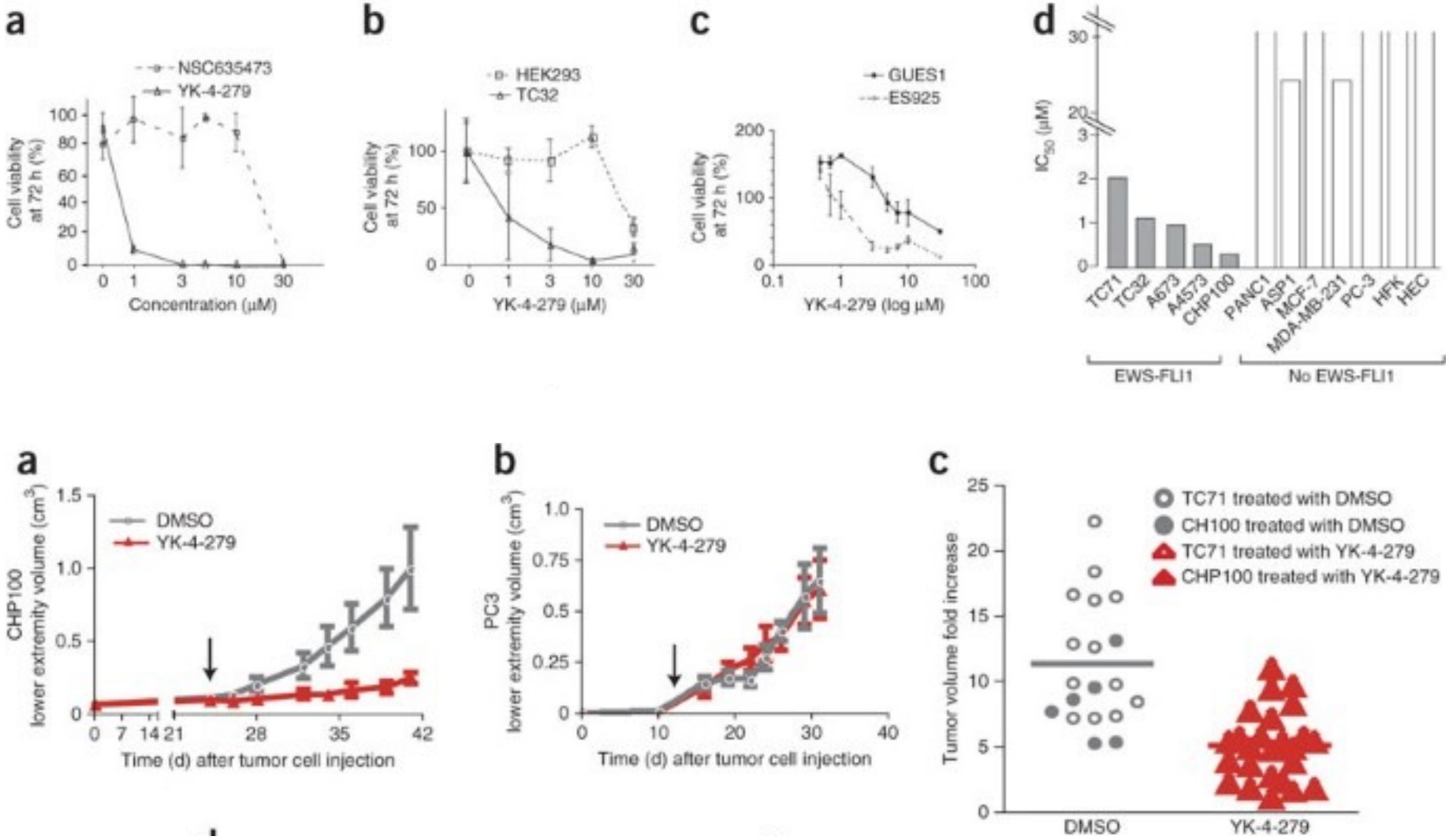


EWS-FLI1 binds to RNA helicase -> ”needed” for transformation

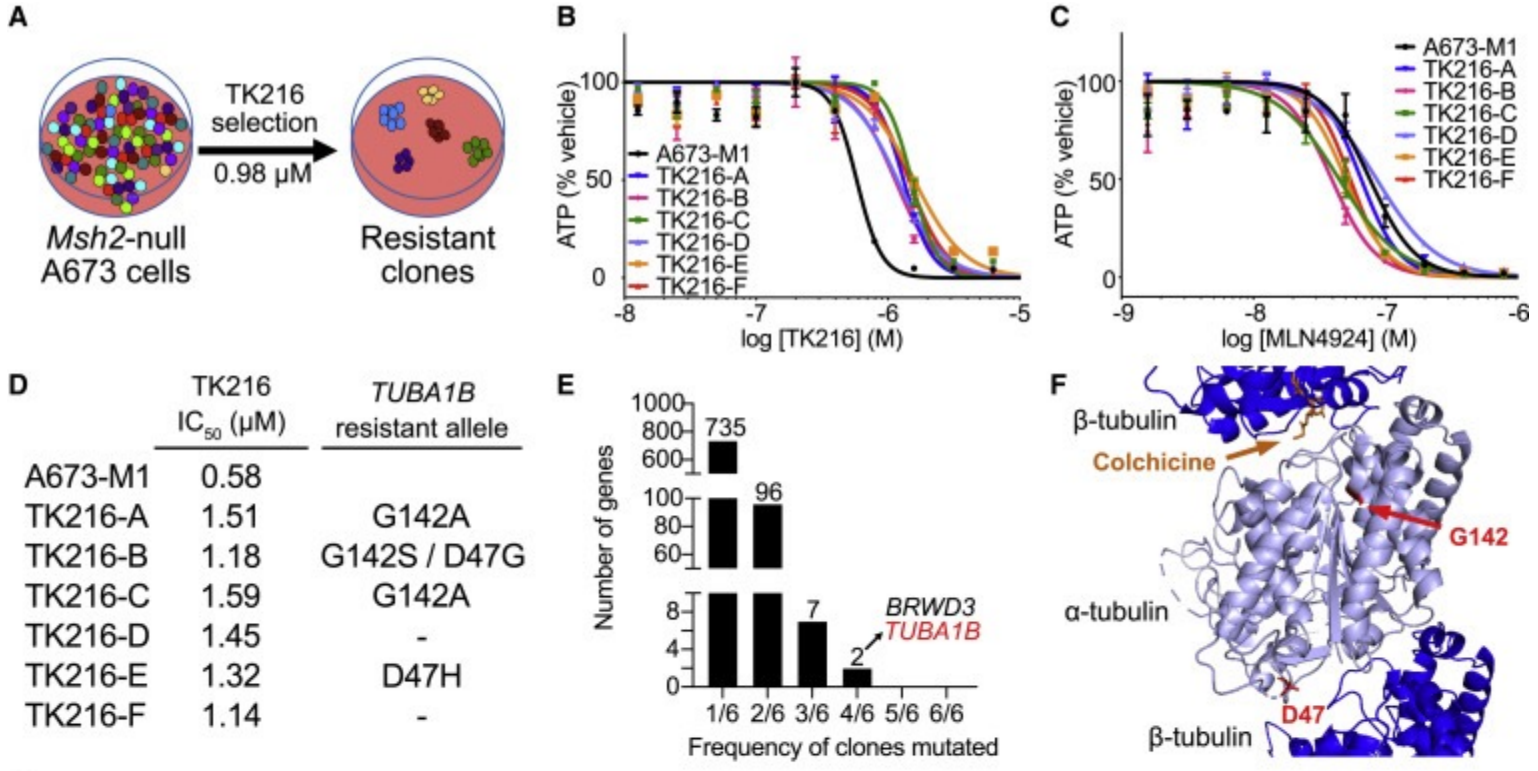


Drug blocks EWS-FLI1::RNA helicase interaction

The EWS-FLI1 inhibitor



The EWS-FLI1 inhibitor



Two approaches for understanding drug mechanism:

1. Mutations that promote resistance
2. Does the drug work when the target is deleted?

The Reproducibility Crisis

1. Reproducibility vs Robustness

Heterogeneity is the norm in cancer and the lab. Embrace this, even if journal reviewers do not.

- Ex. 10 of 13 BRCA mutant lines are sensitive to the new PARPi. Why only 10?

The Reproducibility Crisis

2. The problem with “down” assays

Genetic and chemical perturbation can cause cancer cells to die for numerous reasons – on and off target.

BCR-ABL driven CML. Drug blocks Abl kinase. Does that prove this is why imatinib kills CML cells?

The Reproducibility Crisis

3. Multiple Hypothesis Testing

20,000 genes. At a $p<0.01$, how many genes are expected to be essential by chance.

Example 2: RAS and cancer

MORE THAN

30%

OF ALL HUMAN CANCERS ARE DRIVEN BY MUTATIONS OF

RAS GENES

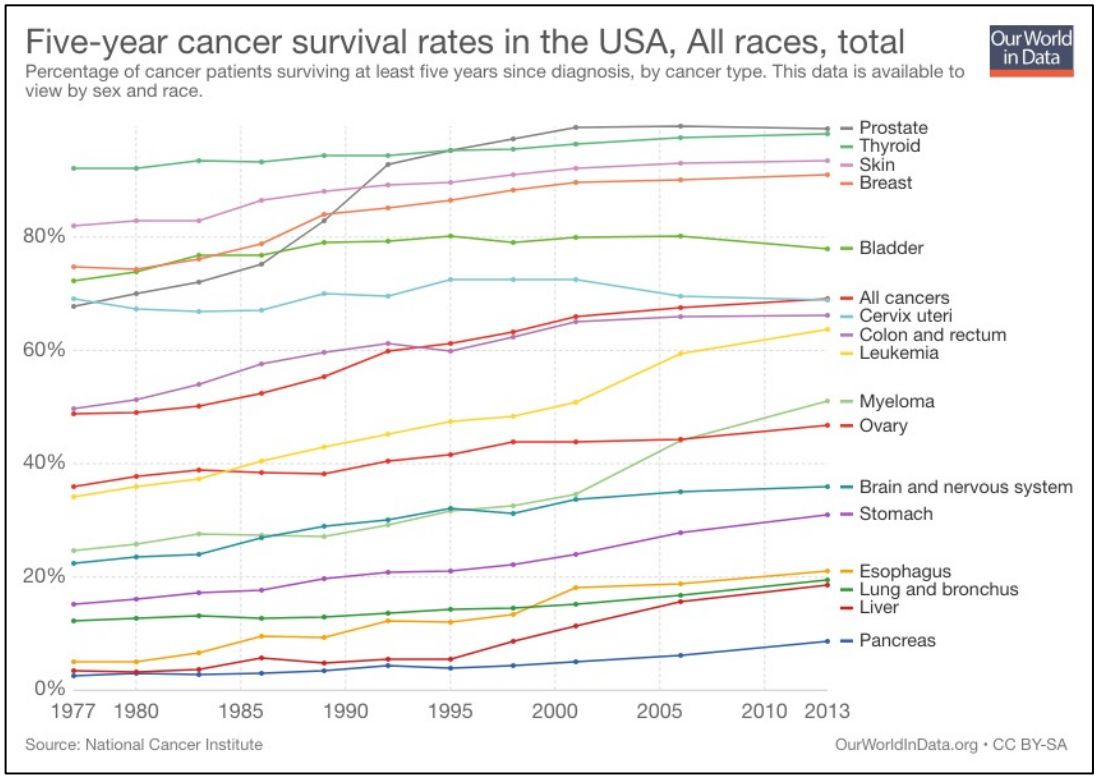
RAS MUTATIONS

IN HUMAN CANCERS

PANCREAS – KRAS	95%
COLORECTAL – KRAS	45%
LUNG – KRAS	35%
AML – NRAS	15%
MELANOMA – NRAS	15%
BLADDER CANCER – HRAS	10%

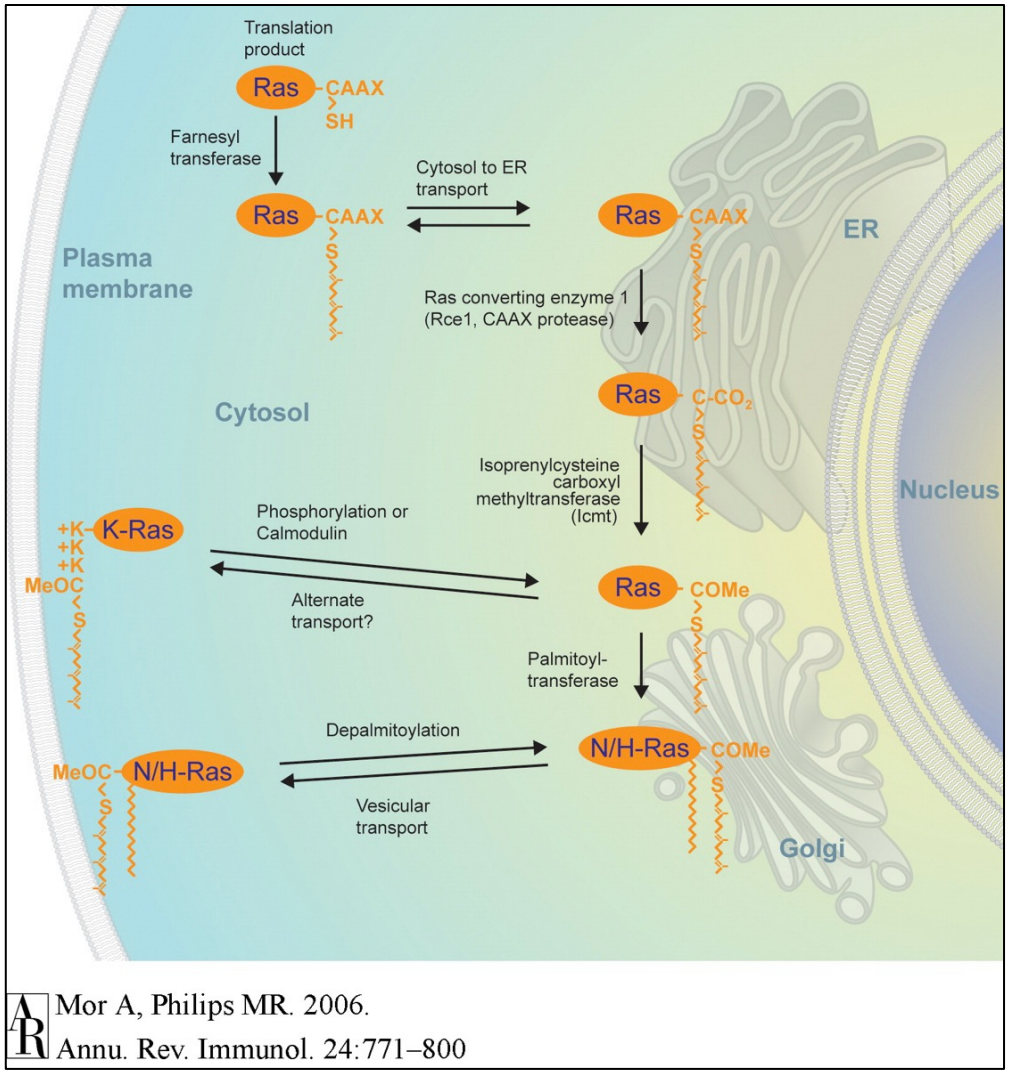
"RAS ONCOGENES ARE THE **WORST** ONCOGENES."

– Dr. Frank McCormick, RAS National Program Advisor



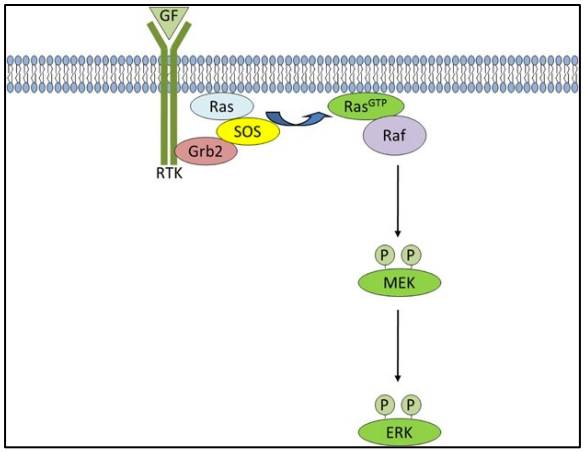
RAS signaling

RAS structure/processing

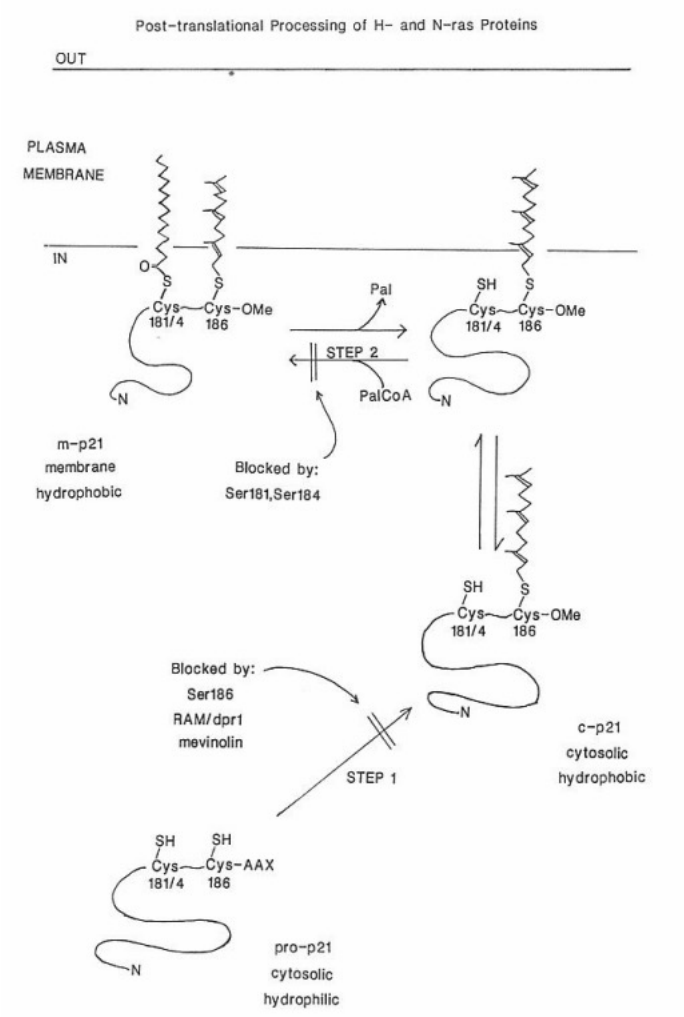


Mor A, Philips MR. 2006.
Annu. Rev. Immunol. 24:771–800

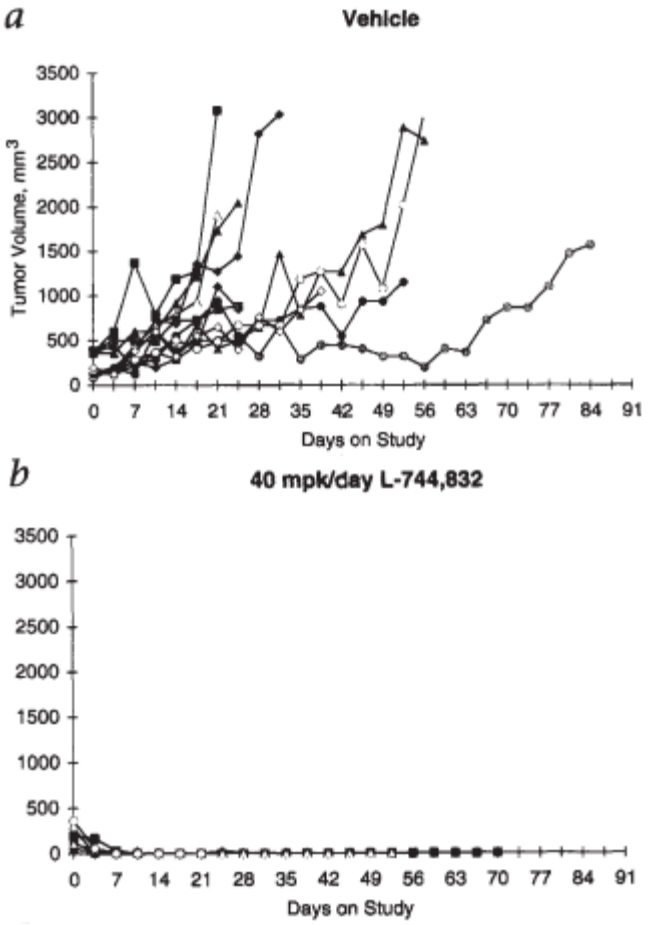
Plasma Membrane



Blocking Farnesylation of RAS

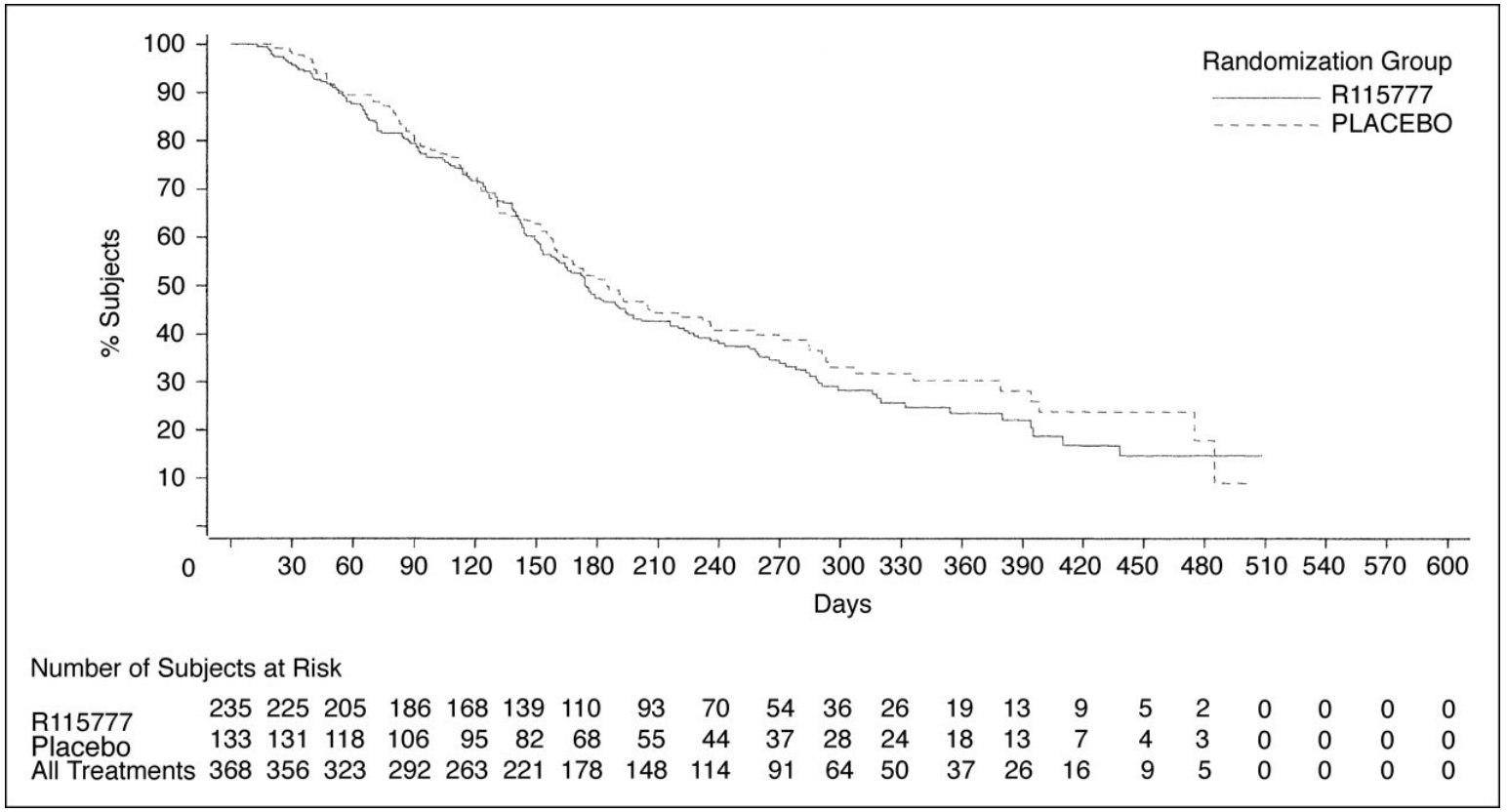


Hancock et al, 1989



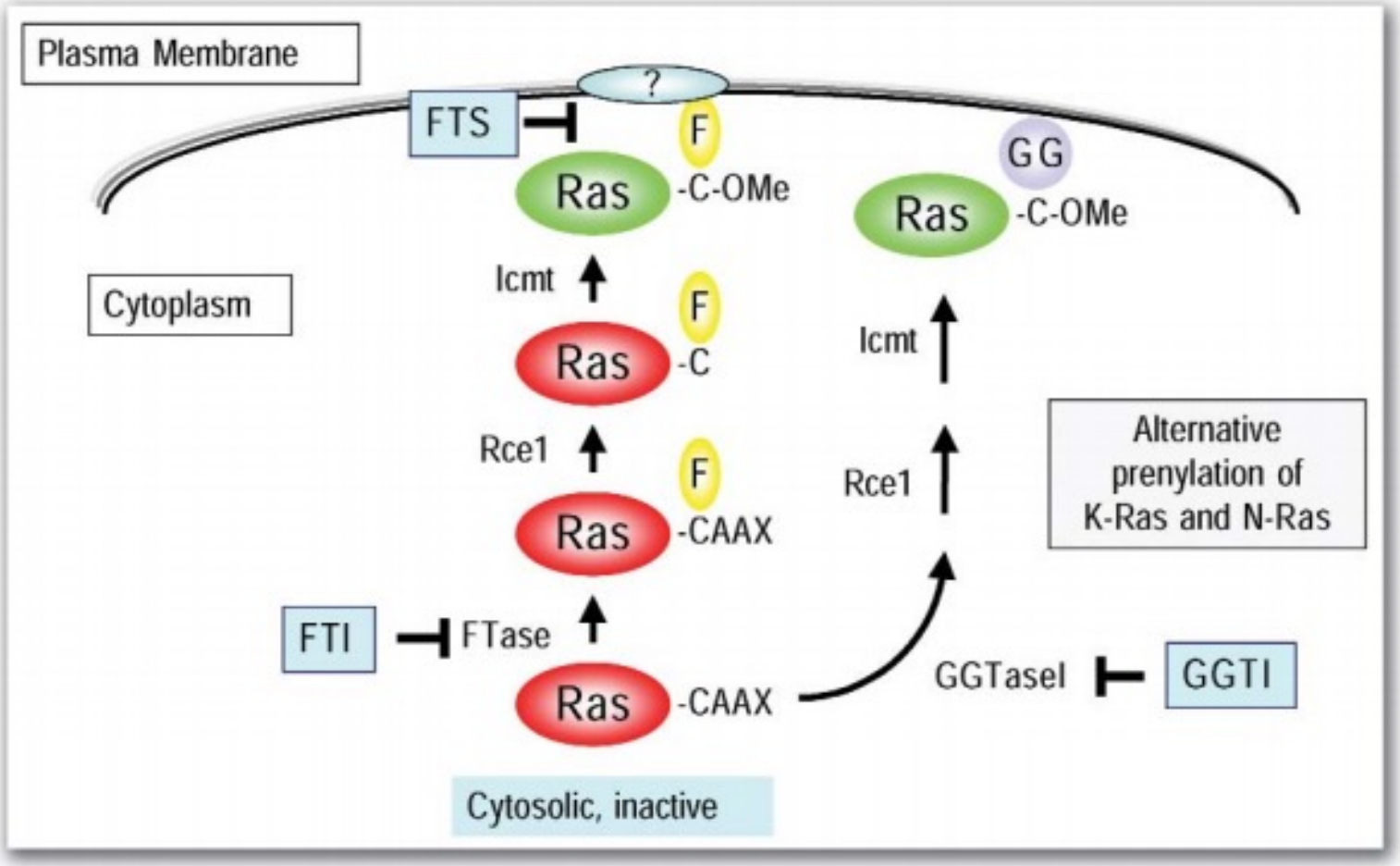
Kohl et al, 1995

Clinical Trials

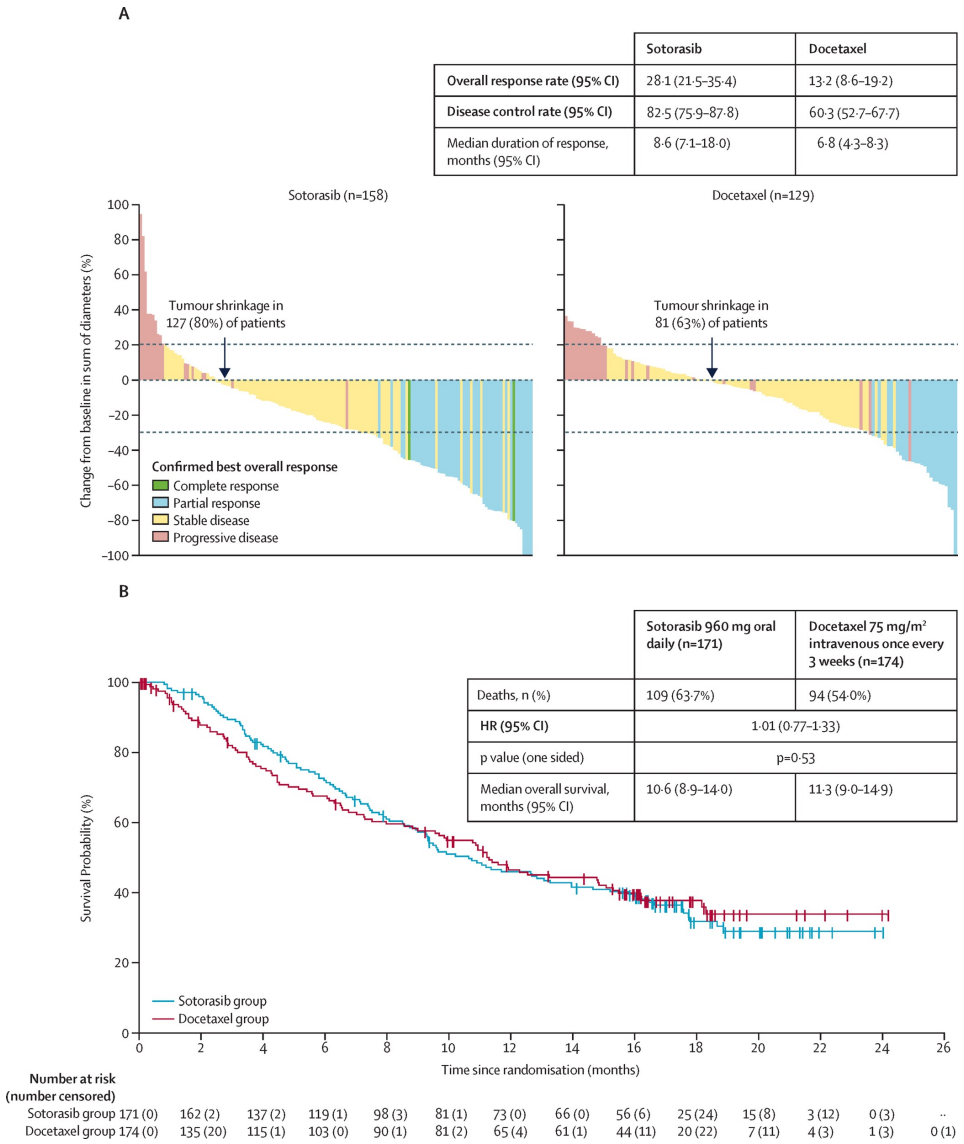


Rao et al, JCO 2004

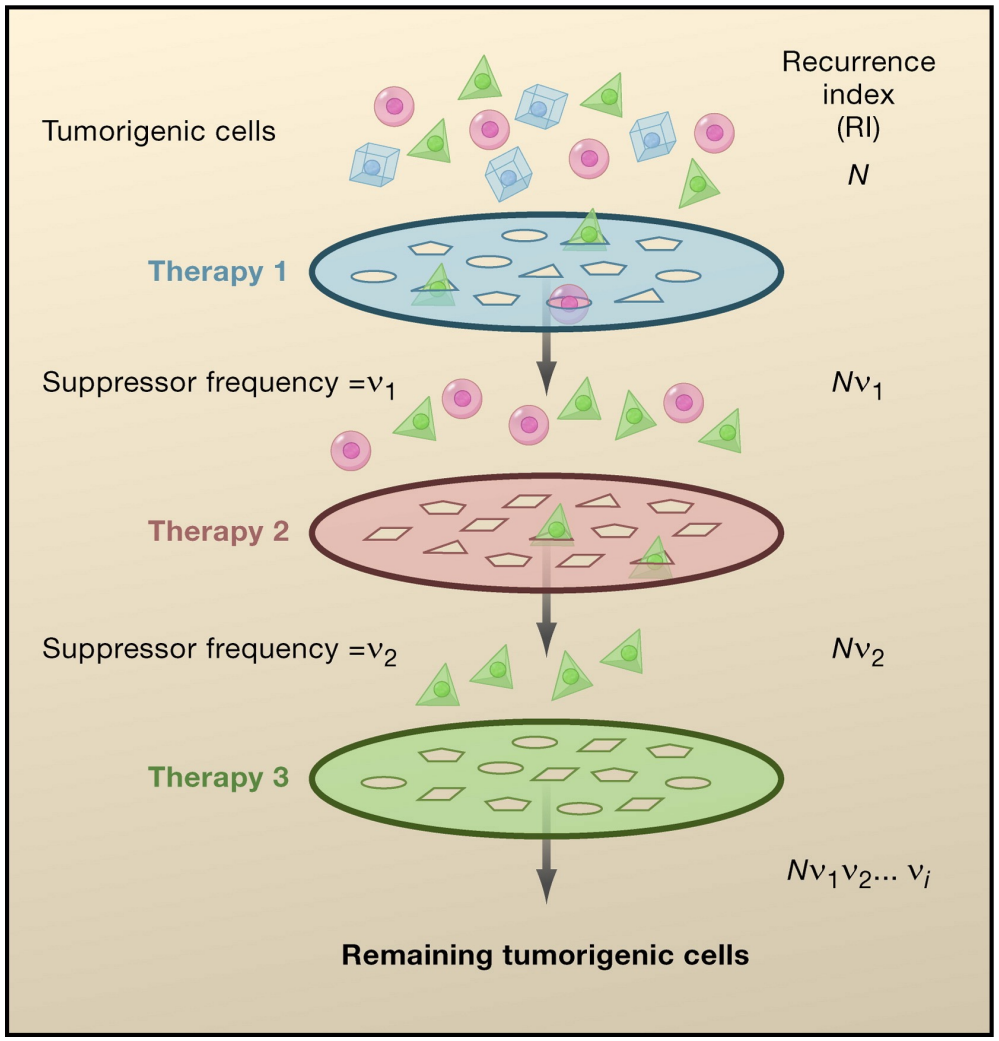
What happened?
De-novo resistance to FTIs



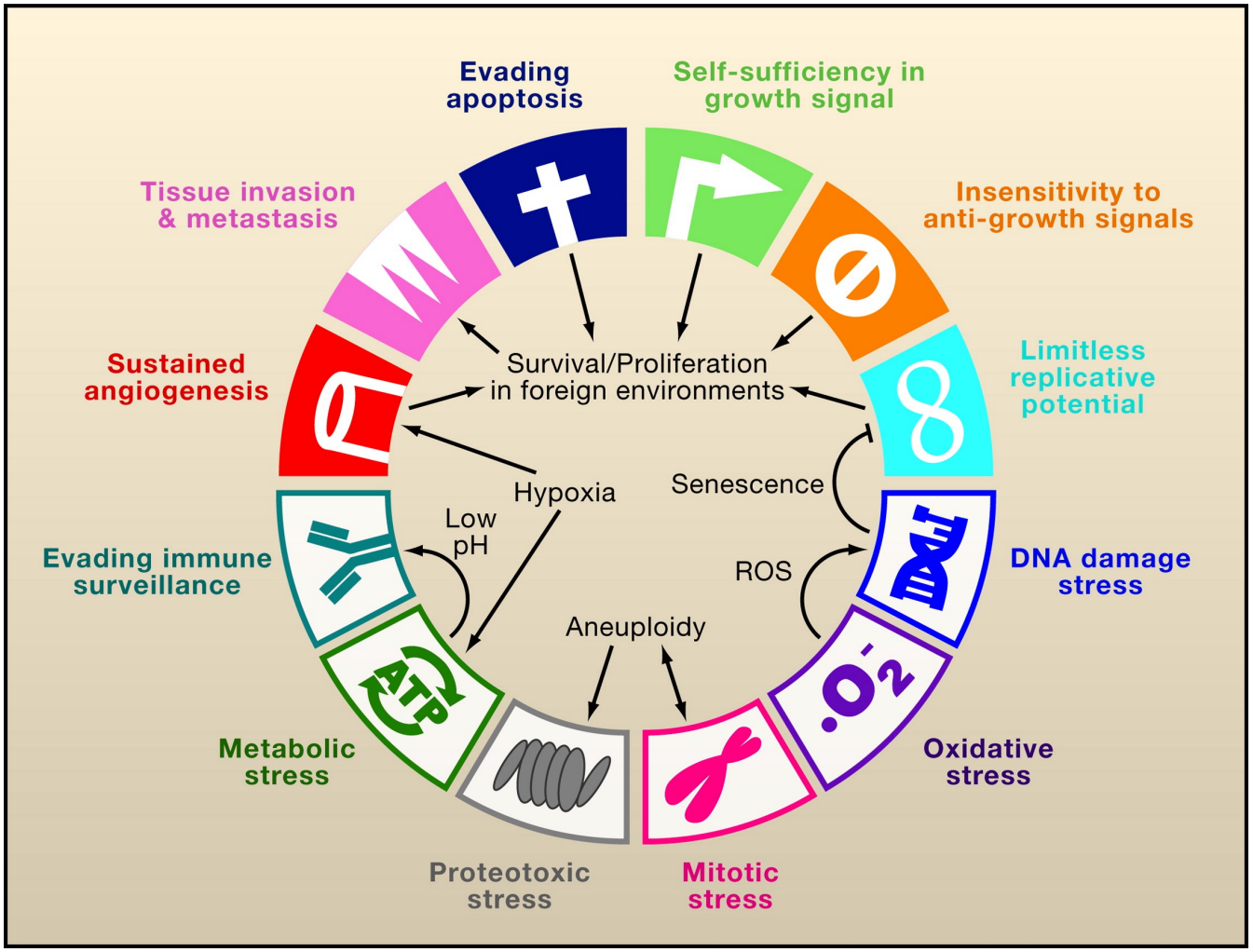
The new RAS inhibitors: 1st trials in KRAS-G12C lung CA



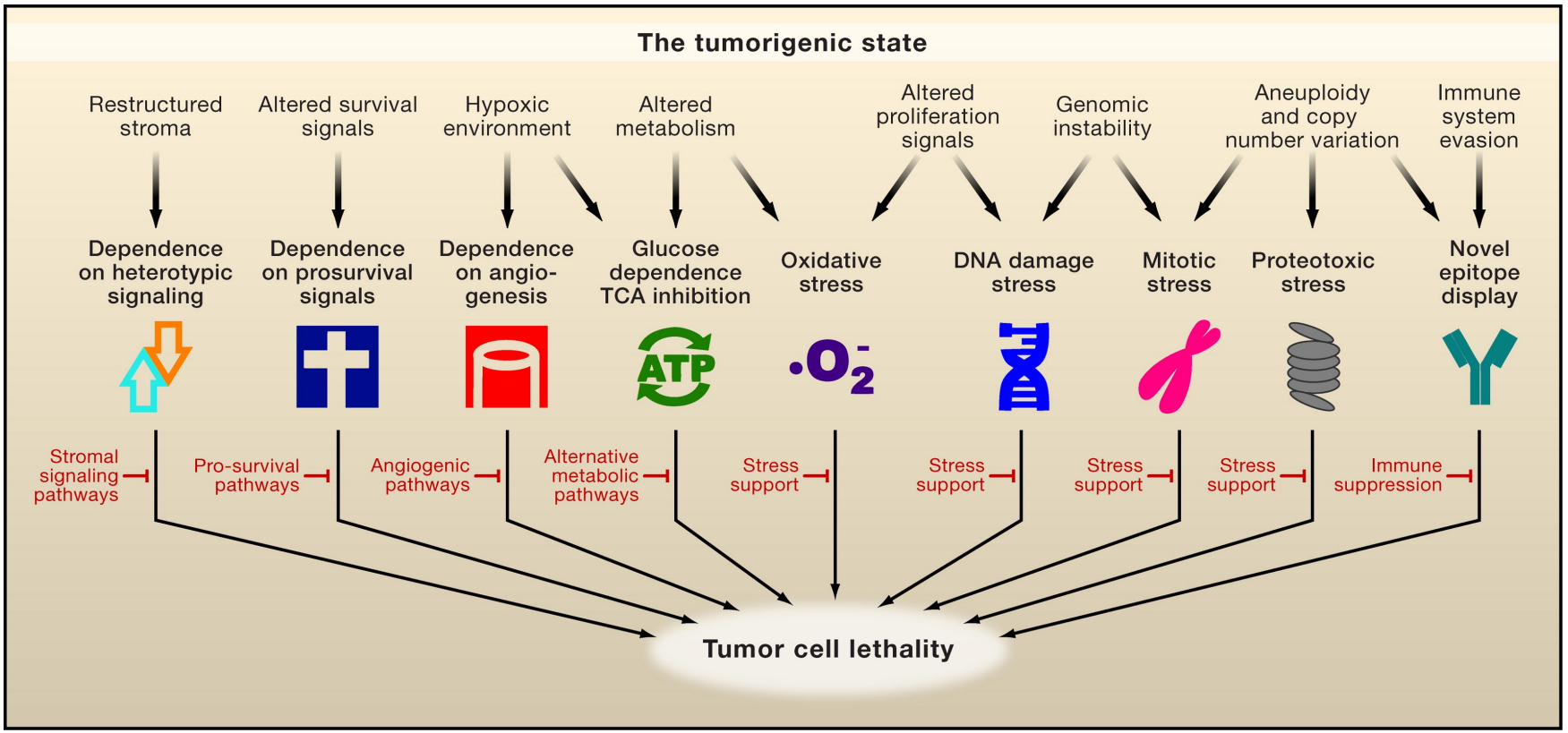
Combination therapy



Moving beyond oncogenes…

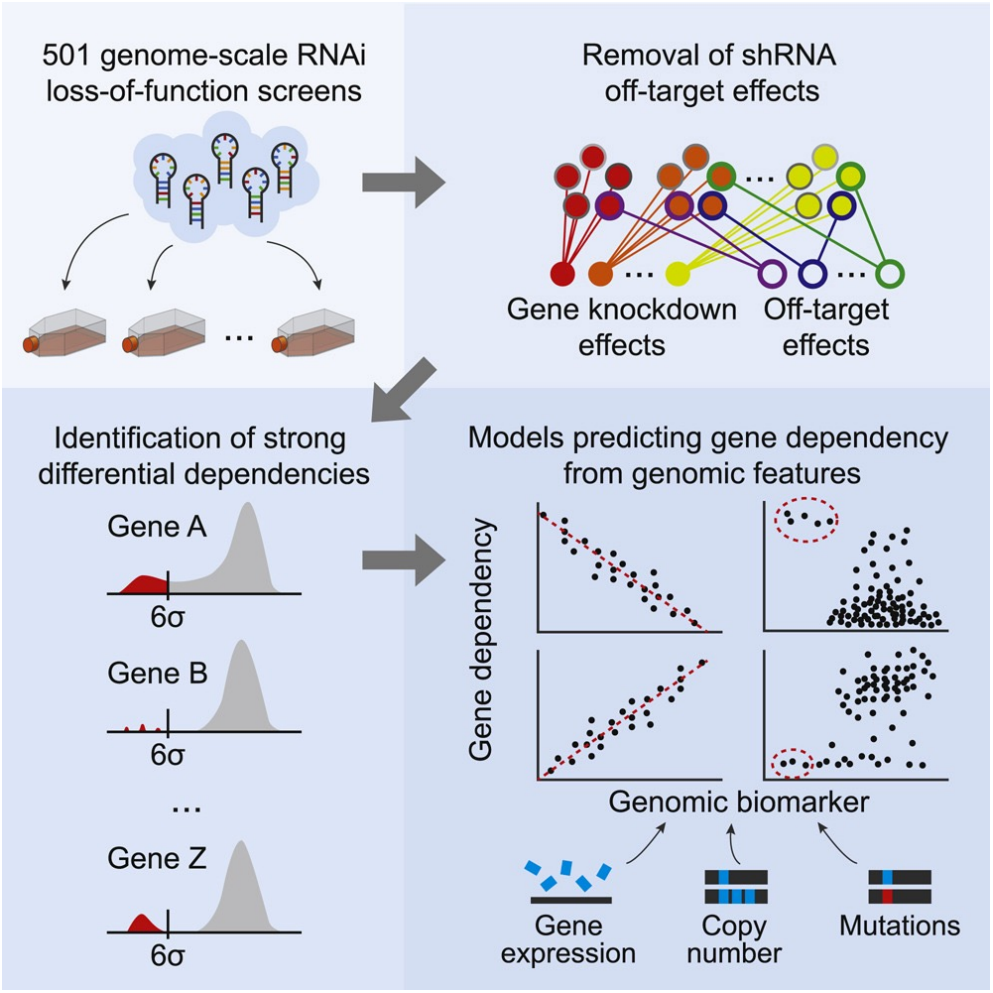


Non-oncogene addiction



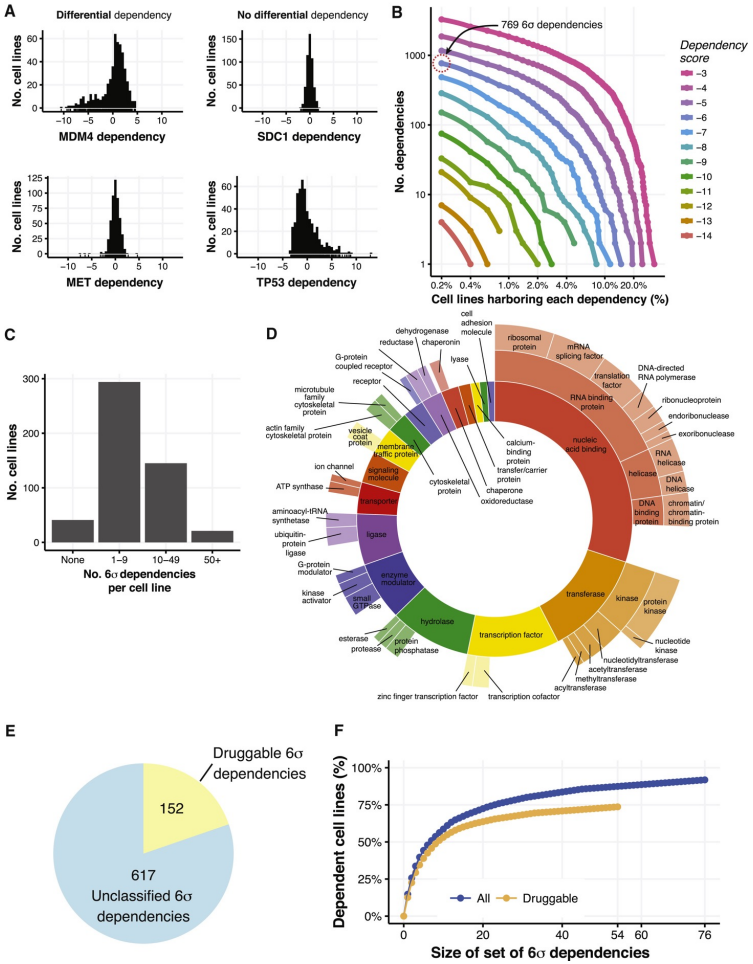
The therapeutic window: Normal cellular stress buffers that cancer cells depend on more than normal cells

Identifying new dependencies



Tsherniak et al, 2017 Cell

700 dependencies across 500 cell lines – where to go from here?



Challenges for Future Cancer Research

1. Understand residual disease

2. The problem with Cell Lines (p53 as an example)
   - design better systems, heterogeneity
   - organoids, PDX, mouse models…

3. More basic cell biology – how do oncogenes work?

4. Design better screens and then follow-up the dependencies with biology

5. Study and understand existing dependencies

6. Use clinical data and clinical samples to think more about mechanism. Can we move beyond profiling patient samples and test hypotheses/do experiments?

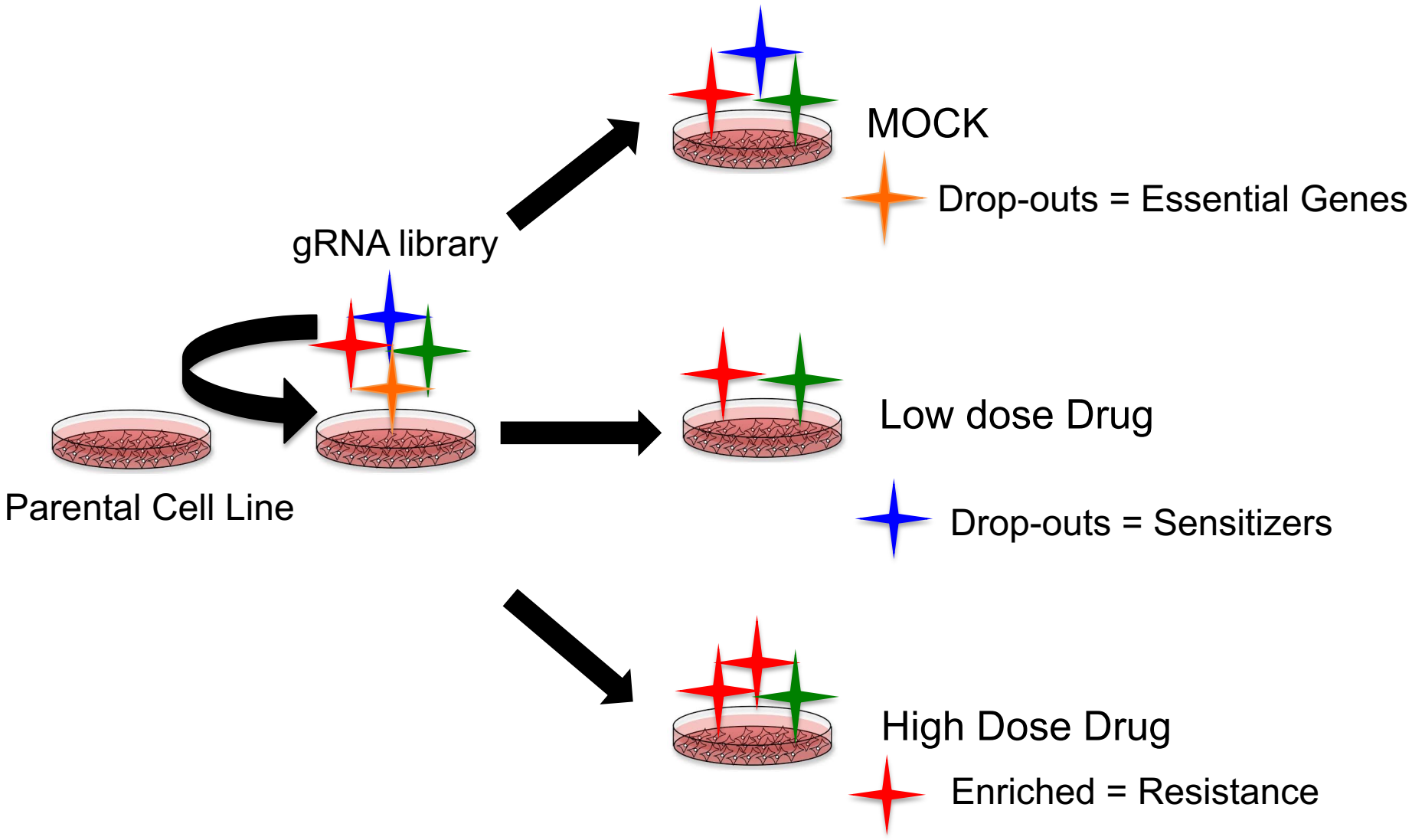
7. Think hard about necessity vs. sufficiency

8. Rescue dependencies in various ways…

Challenges for Future Cancer Research

“There has been a trend, especially in papers in high-profile journals, towards making far-reaching claims in an attempt to paint a seemingly complete picture that incorporates both new mechanistic insights and the physiological or clinical relevance of a given set of findings. The field would be better served if papers claimed less, but provided more lines of corroborating evidence in support of their conclusions. “ – Bill Kaelin

Schematic of Genetic Screen



Blind Spots of RNAi based screens

1. Very sensitive to levels of DICER, other siRNA processing enzyme
2. Incomplete knockdown/too good of a knockdown
3. Off-target effects
4. Inflammatory/stress response

Blind Spots of CRISPR/Cas9 screen

1. Amplified genes!
2. Extent of single/double cutting
3. Off-target effects
4. Not good for studying DNA repair
5. Not all guides work well in all cell lines (SNPs)

Blind Spots of CRISPRi screen

1. Alternate promoters
2. Not all guides work well in all cell lines (SNPs)
3. Epigenetic regulators?
4. Incomplete knockdown/too good of a knockdown