

Duesberg

Targeting cancer aneuploidy:

Light at the end of the tunnel?

Today's cancer targets: shoot the horses!

Horses:

- ◆ Oncogenes

- EGFR,

- Her2,

- Abl,

- Raf

- alk

- Btk

- Etc.



- ◆ Oncogenes and tumour suppressor genes drive the development of tumours

Will Targeted Drugs Hit the Wall Soon?



- 1998 Her-2, Herceptin etc
- 2001 Bcr-abl, Imatinib etc
- 2003 EGFR, Gefitinib etc
- 2003 Proteasome, Bortezomib
- 2004 VEGF, Bevacizumab
- 2006 HDAC, Vorinostat
- 2007 mTOR, Temsirolimus
- 2010 Provenge
- 2011 anti-CTLA4
- 2011 ALK, Crizotinib
- 2011 B-raf, Vemurafenib
- 2012 Hedgehog, Vismodegib
- 2013 Btk, Ibrutinib
- 2014 PI3-Kδ, Idelalisib

“There are more paths to developing tumors than there are stars in the sky” R Weinberg

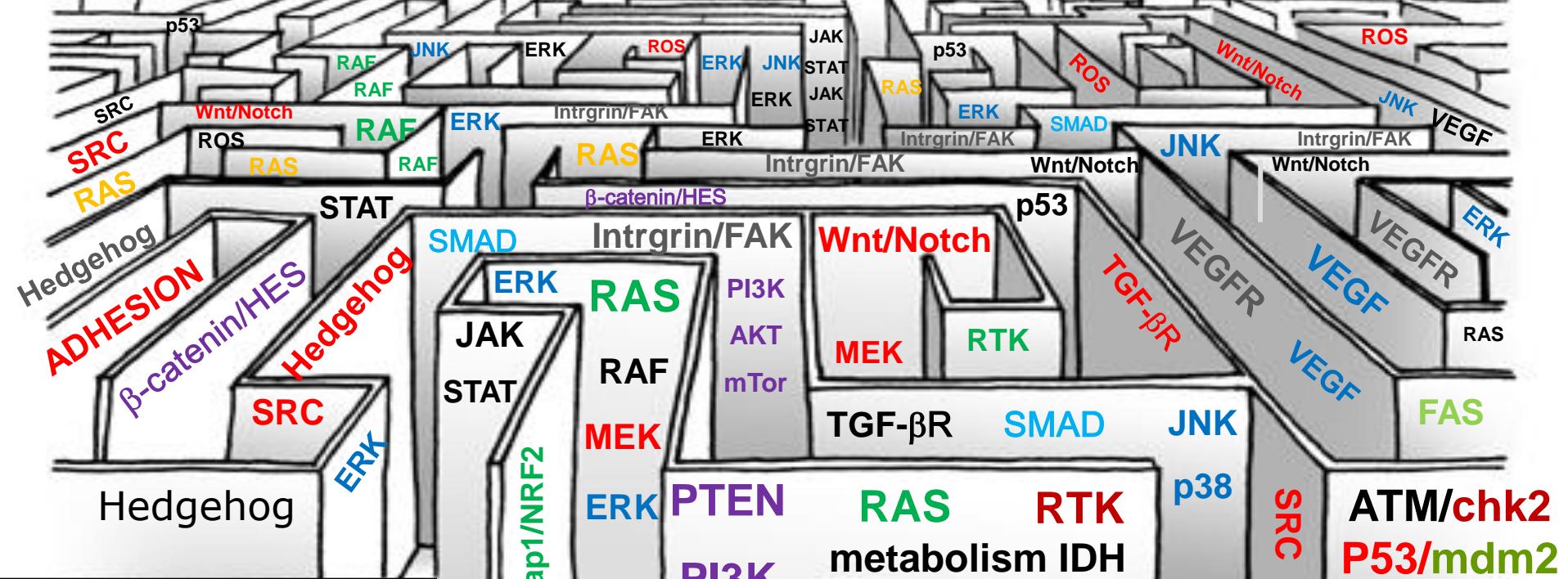
Galaxy 200 billion
Universe 3,000 million billion



CML, APL, Her-2
(Ovarian, NB, etc)

Pancreas
(k-ras p53 p16 smad4)

Breast/Lung/Colon
(EGFR ALK mTOR)



Personalised Medicine:

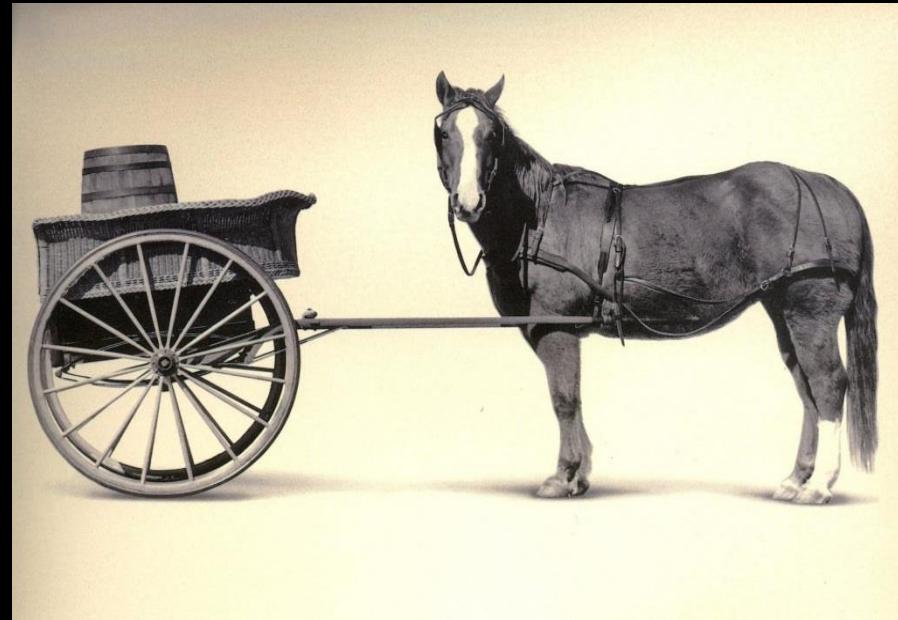
1. Biomarkers (genomics)
 2. Drug Combinations
 - pathways knowledge
 - Intuitive deductions

Future: target the carts?

- ❖ Oncogenes

Carts:

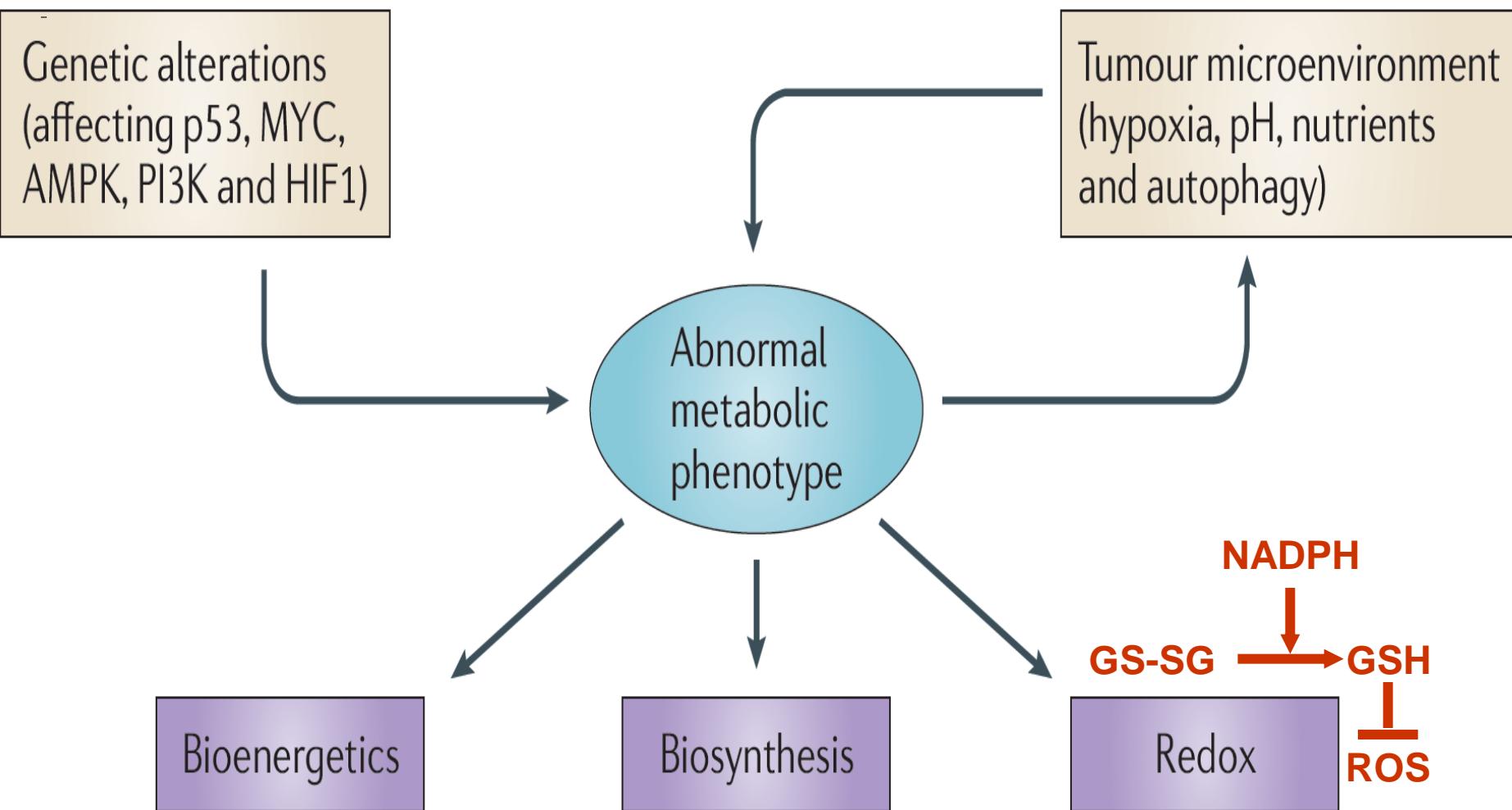
- ❖ Immune
- ❖ Metabolism
- ❖ Aneuploidy



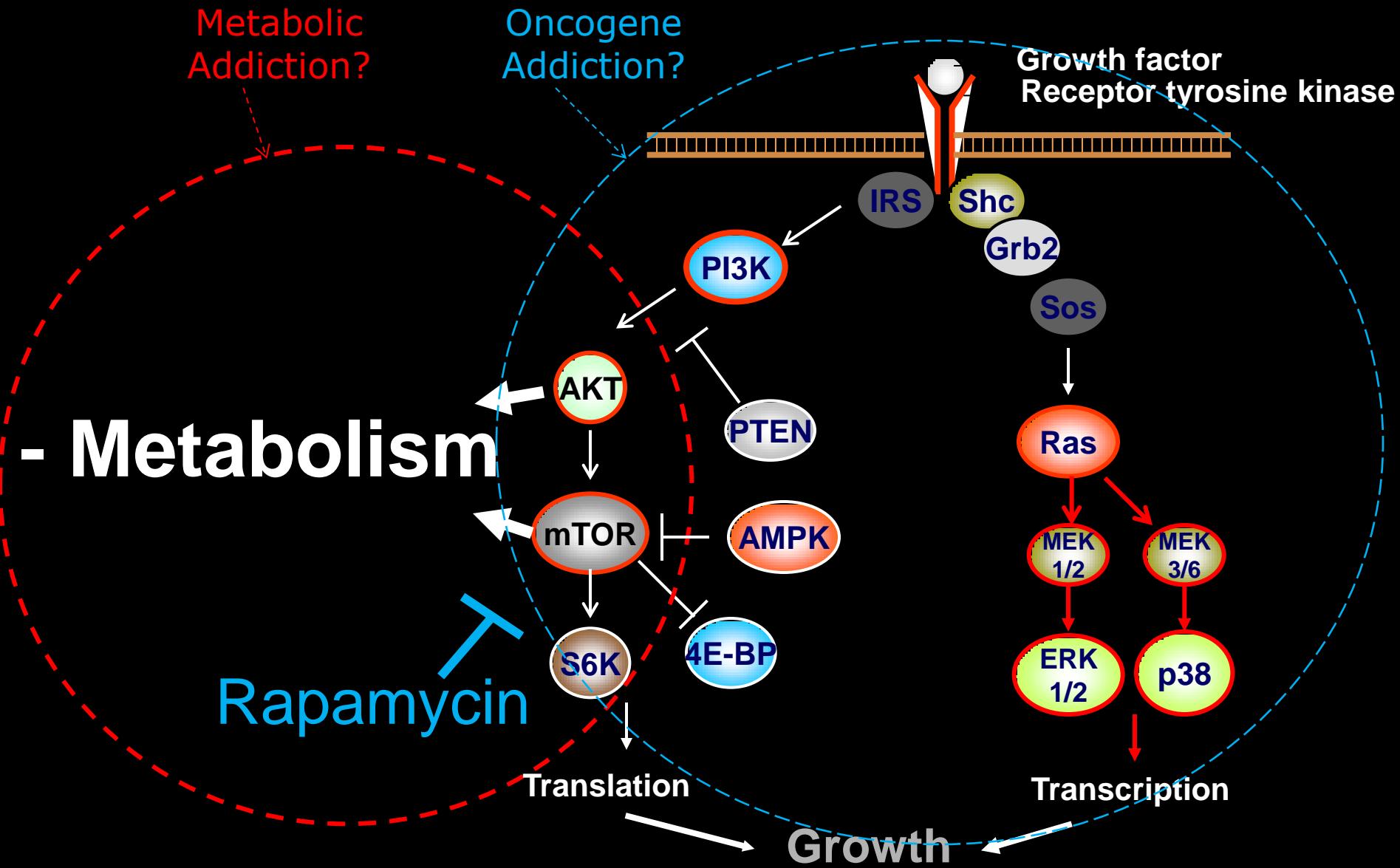
- ❖ The cart is the transformed state of the cell as a consequence of the actions of oncogenes and tumour suppressor genes

Carts are INDEPENDENT of oncogenes & TSGs

Determinants of the Tumour Metabolic Phenotype



Are Tumours Addicted to Oncogenes or Metabolism?



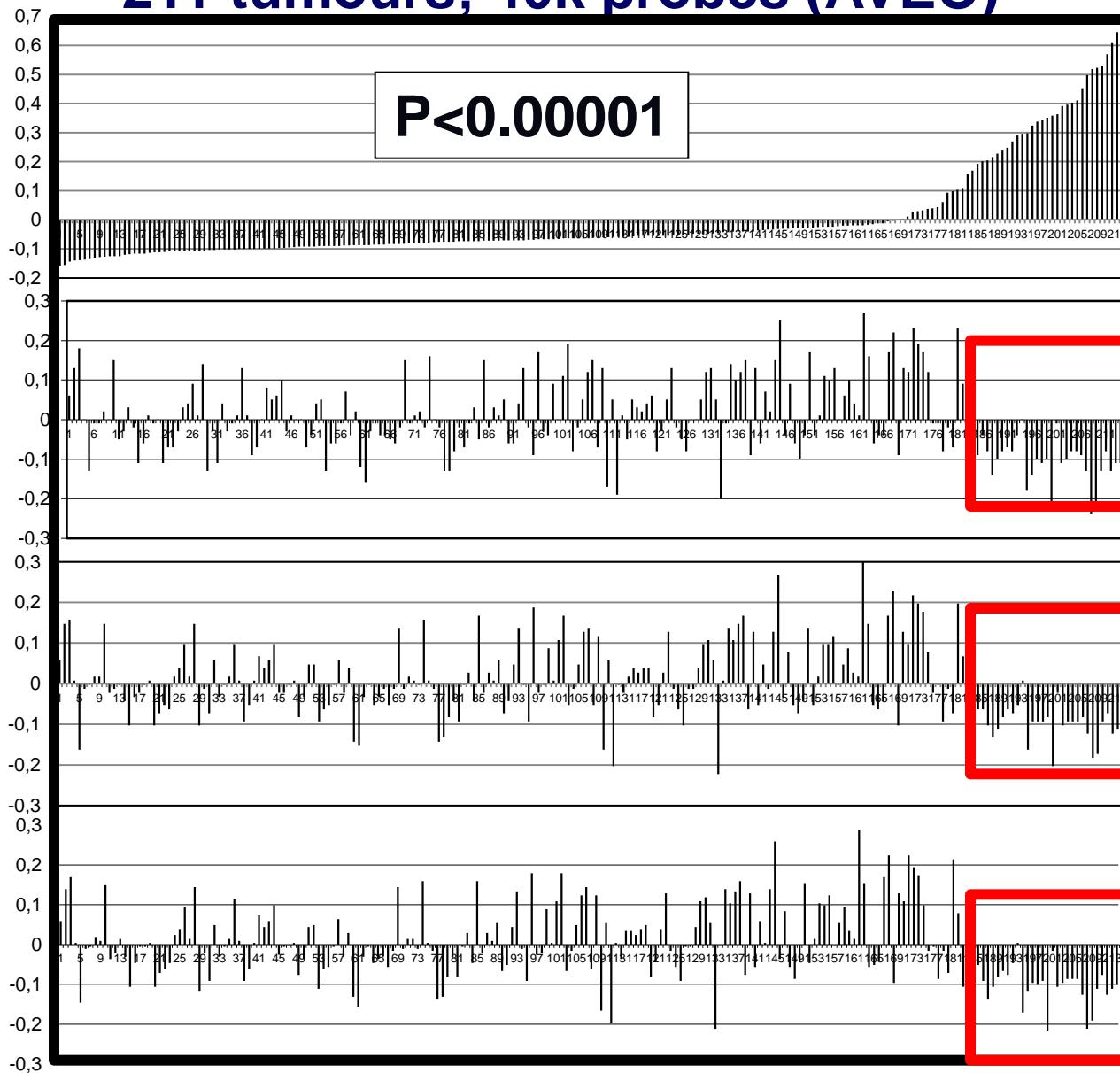
CPT1C Expression Correlates With Resistance to Rapimycin 211 tumours; 40k probes (AVEO)

PI3 Kinase
Index

Cpt1c
probe 1

Cpt1c
probe 2

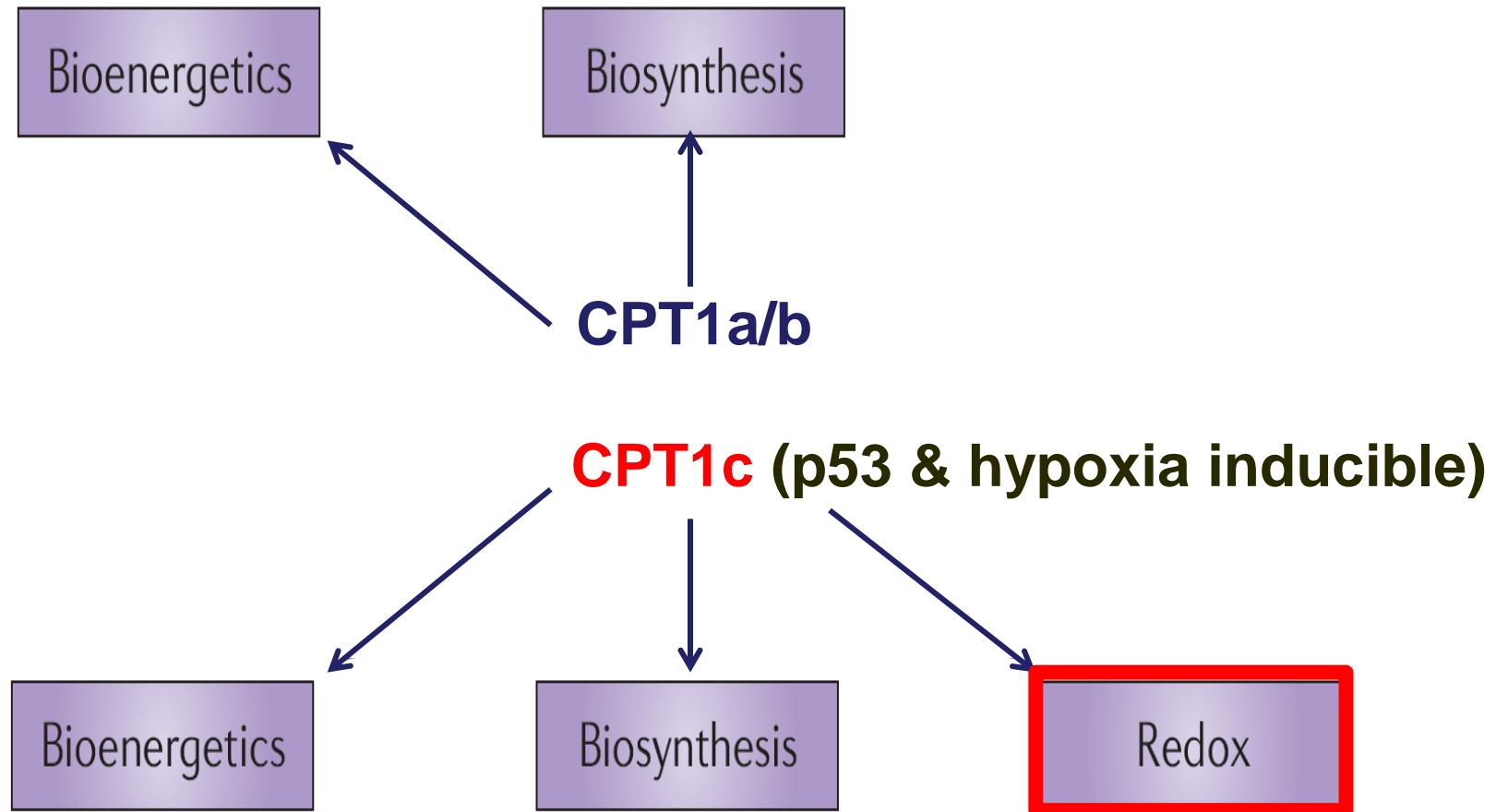
Mean
Cpt1c



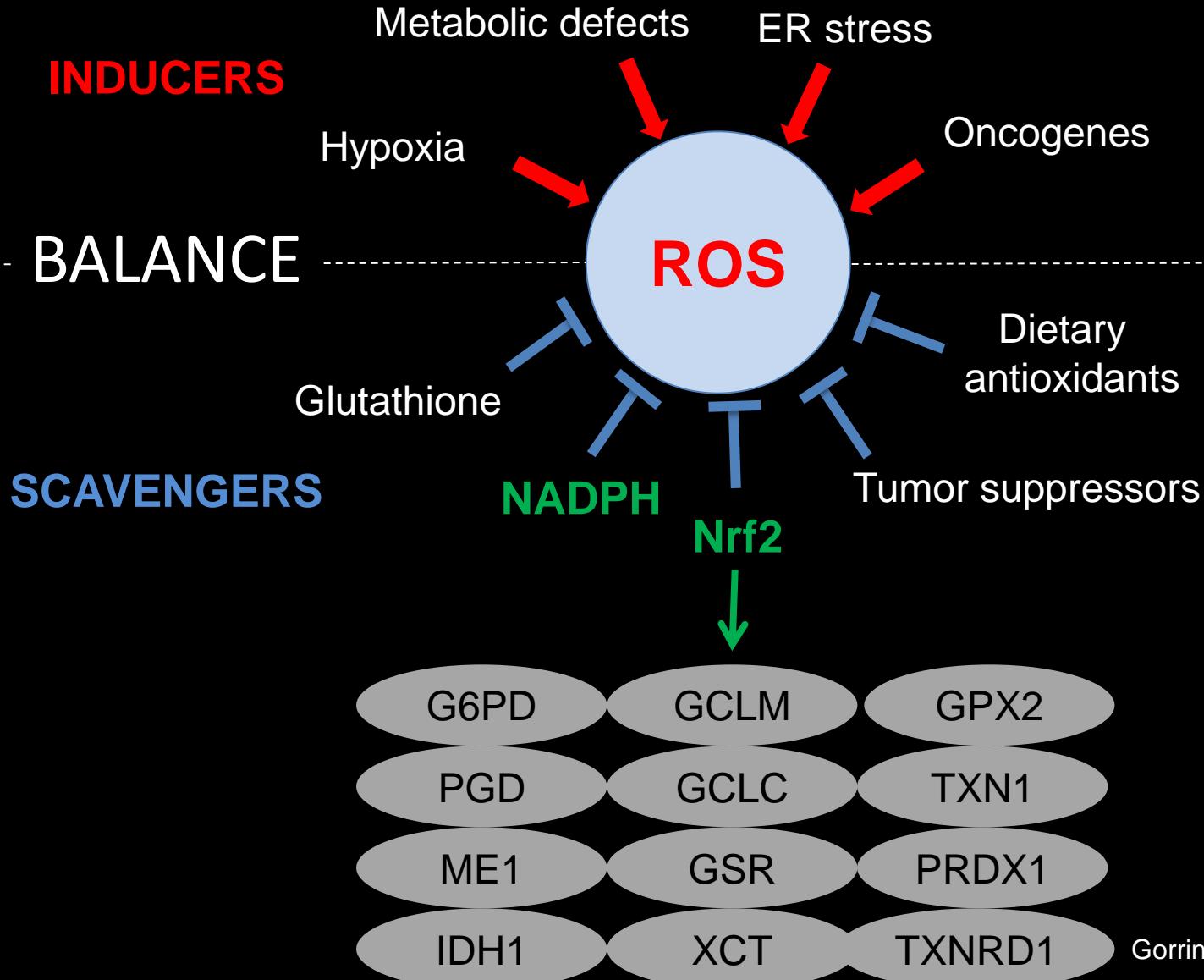
No correlations with cpt1a/b!!

Zaugg et al G&D 2011

The Role of CPT1c in Tumour Metabolic Adaptation

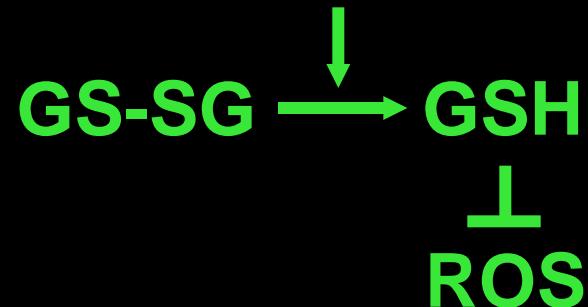


Inducers and Scavengers of Reactive Oxygen Species:



Gorrini et al Nat Rev Drug Dis 2013

Potential Sources of Reducing Equivalents (NADPH)



Pentose Phosphate Shunt

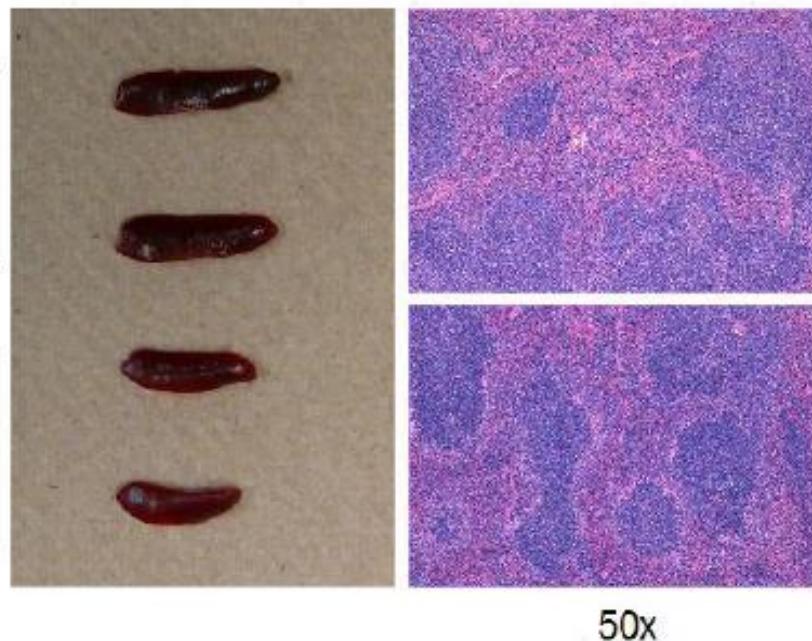
Glutaminolysis

→ Isocitrate Dehydrogenase 1/2*

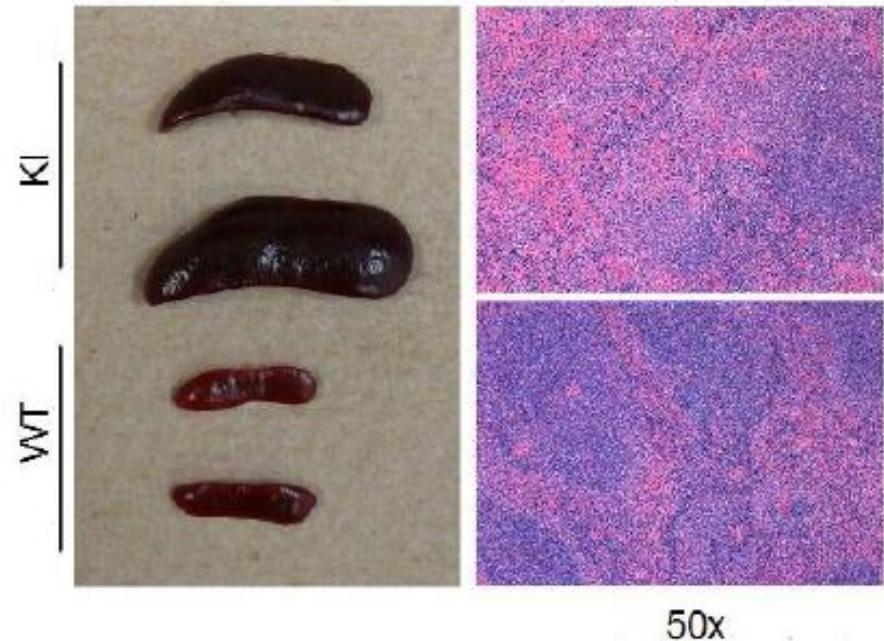
*IDH1/2 mutated in GBM, glioma, AML, AITL, Chon-Sa, Chol-Ca

LysM-Knock-in Mice Exhibit Age-dependent Splenomegaly & Decreased BM Cellularity Extra-medullary Hemopoiesis, Increases in progenitors - similar to Myelodysplastic Syndrome

young mice



older mice



Preliminary Results on AG-221 mIDH2 Inhibitor Ongoing Phase 1 Clinical Trials on **Refractory** AML

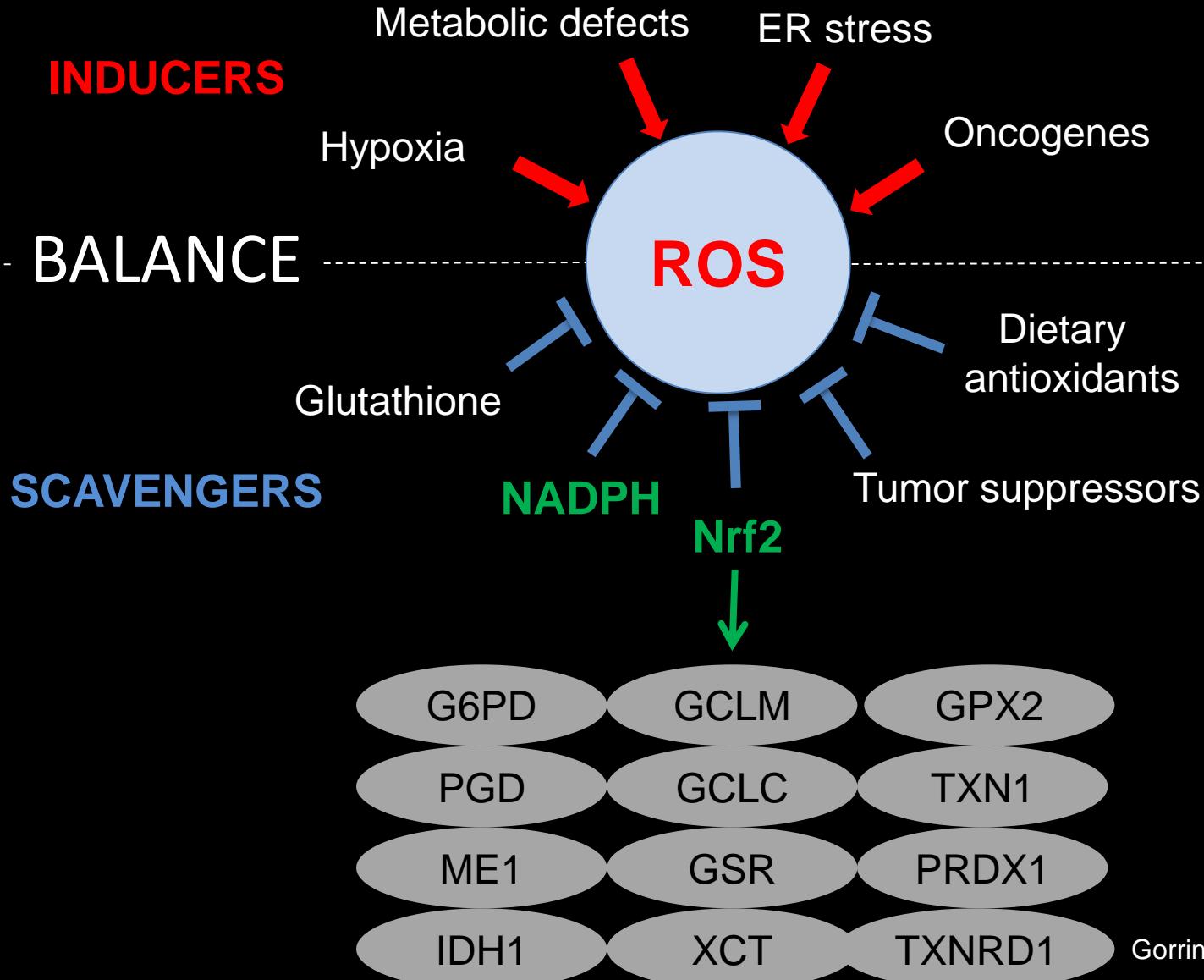
	2HG	
Complete remission	9	↓
Partial remission	5	↓
Progression disease	6	↓
<hr/>		
Total	20	

Drug well tolerated

No discontinuation due to adverse events

Not reached MTD

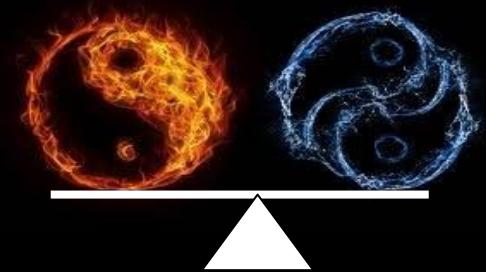
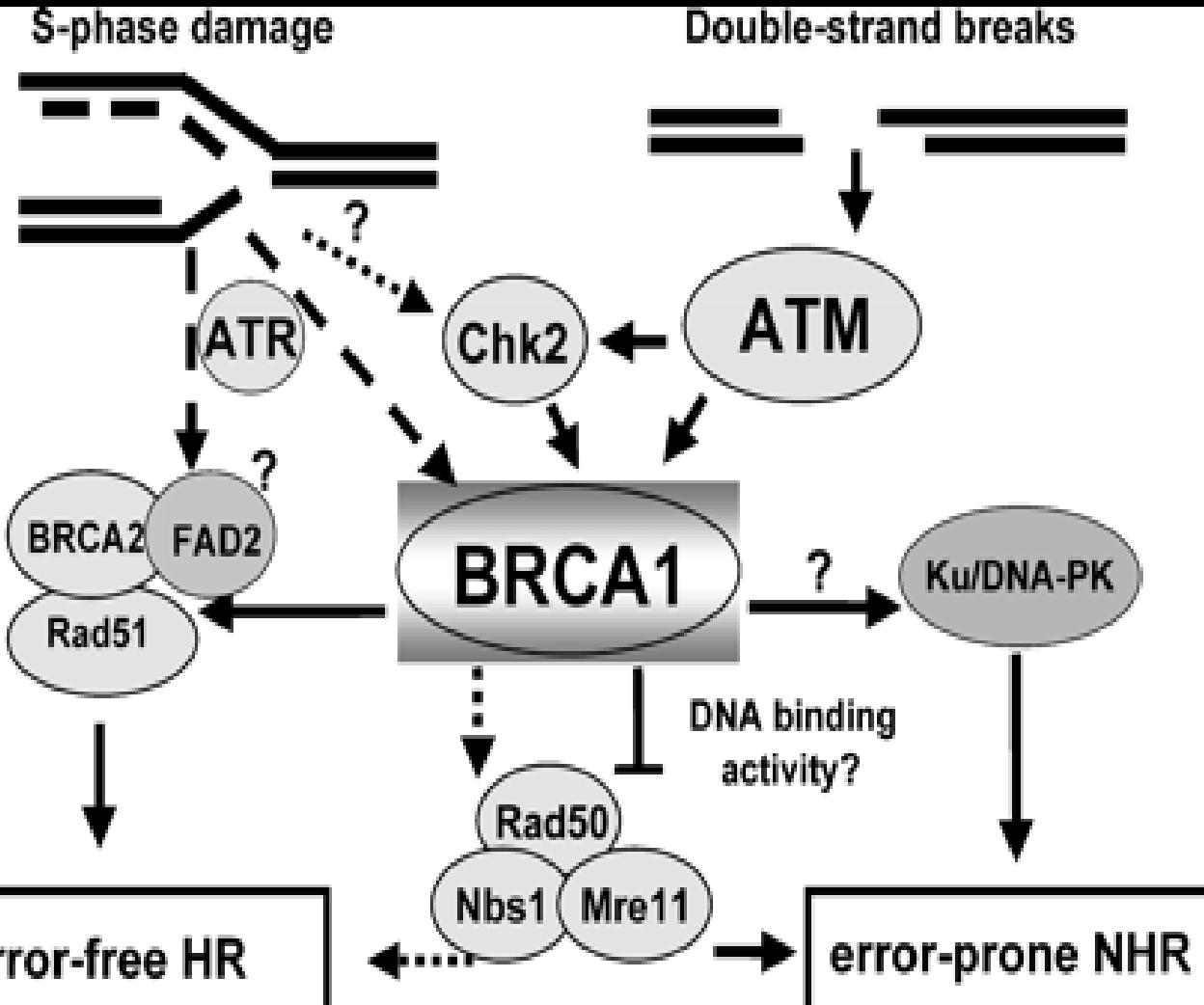
Inducers and Scavengers of Reactive Oxygen Species:



Gorrini et al Nat Rev Drug Dis 2013



Can the regulation of ROS levels explain BRCA1 carriers mainly only develop Breast and ovarian cancers?



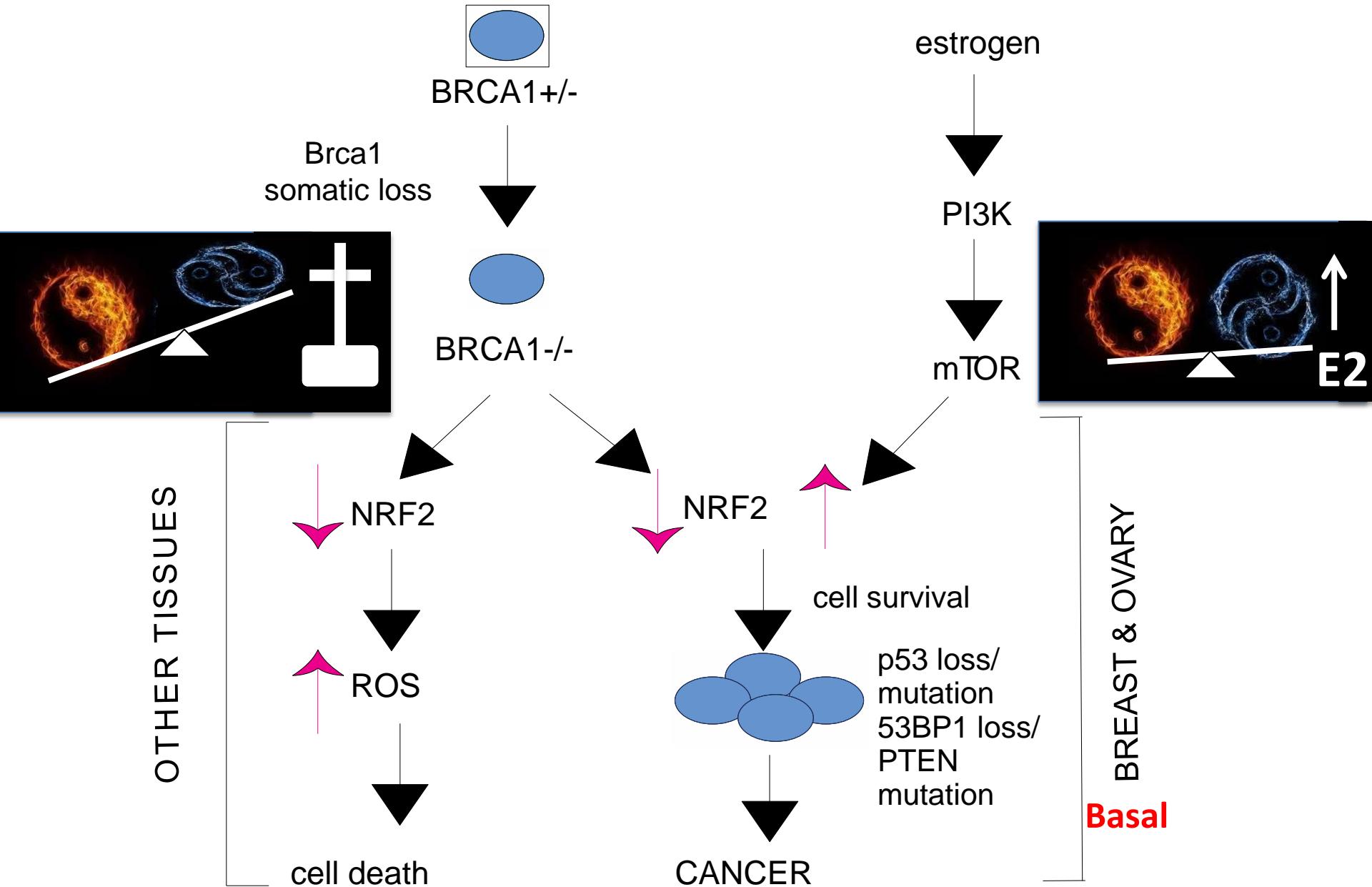
life time risk

70% breast

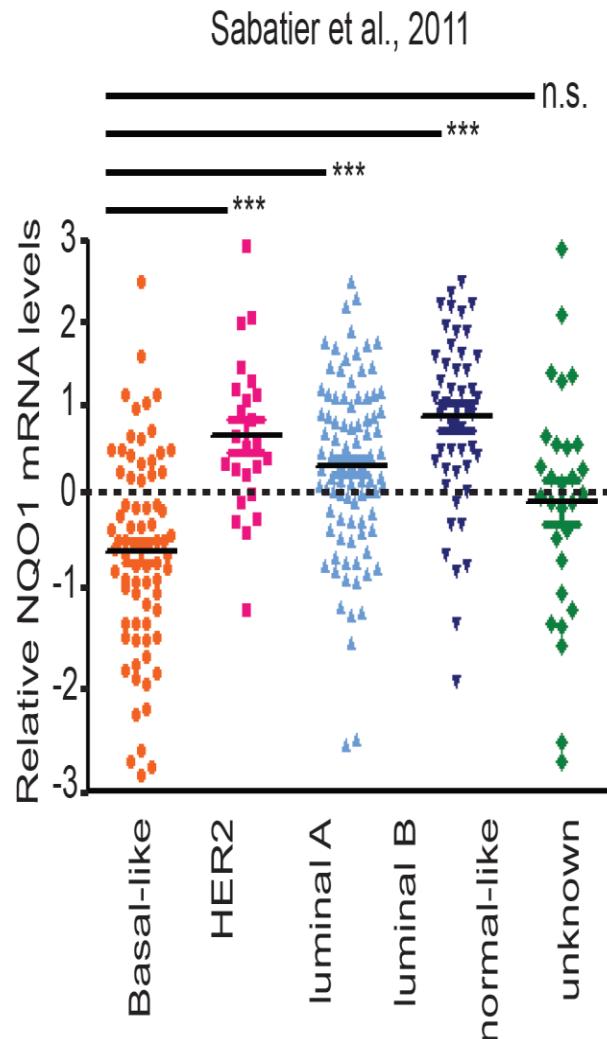
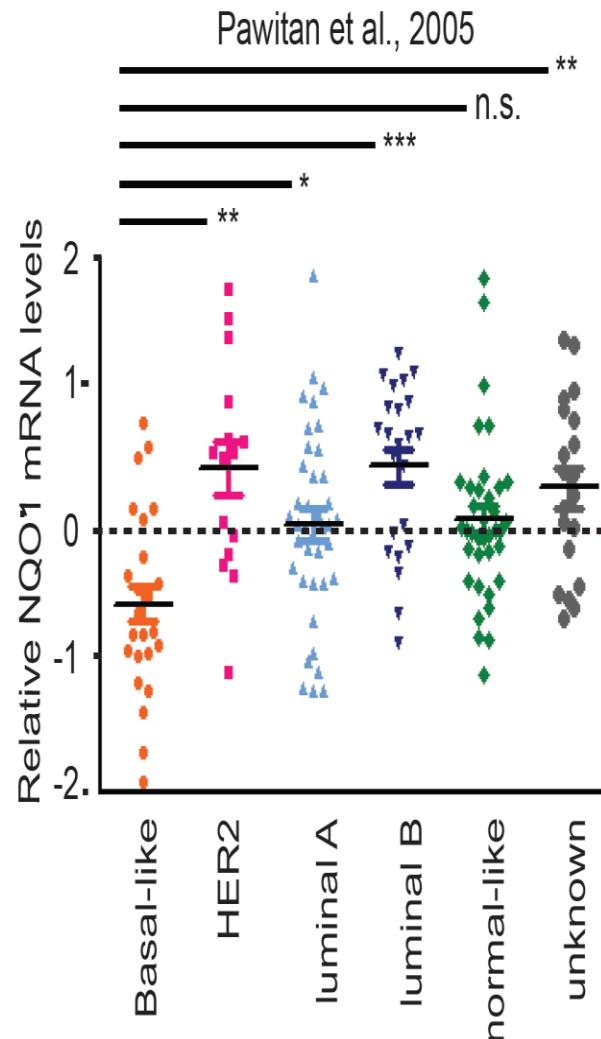
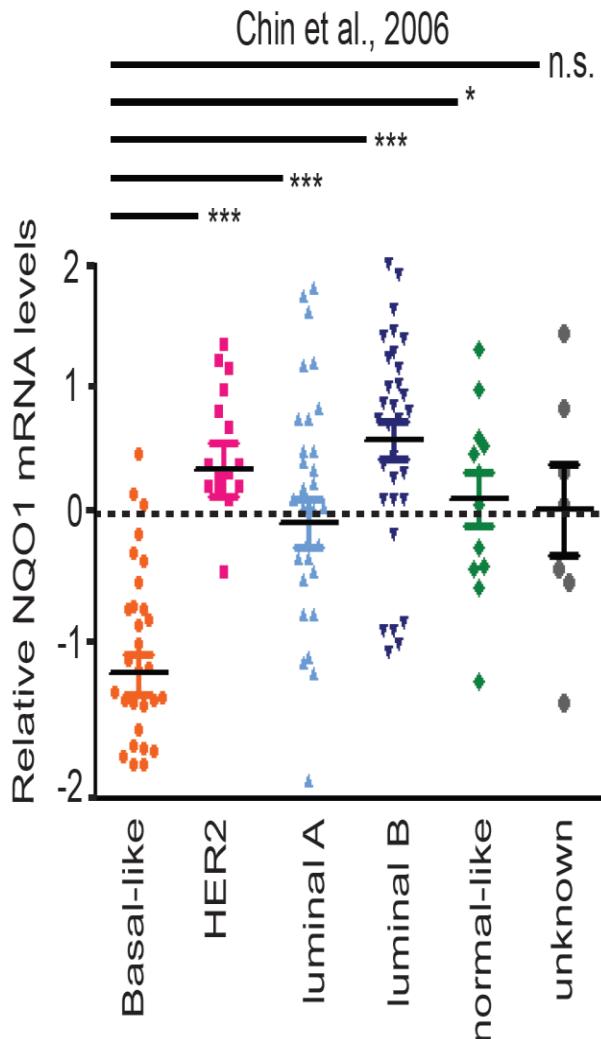
40% ovarian

15% prostate

Estrogen-controlled NRF2 activation in BRCA1-related tumorigenesis



Basal-like Breast Cancers Express Lower Levels of Nrf2 Target Gene NQO1



Percent Involvement of PTEN, BRCA1, p53 Breast Ca Subtypes

	LumA	LumB	Her-2	Basal	Ovarian
PTEN *	15	27	19	42	40
BRCA1	3	2	3	24	18
P53	12	30	72	77	96
At least 1	27	47	78	91	96
5 years survival	90	70	50	40	20

Nature. 2012 Sep 23. doi: 10.1038/nature11412.

*Nuclear PTEN controls homologous recombination mediated DNA repair and sensitivity to DNA damage Bassi et al Science 2013

Oxidative Stress

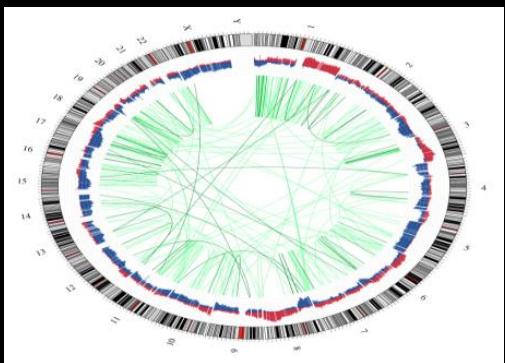


DNA Repair



Aneuploidy

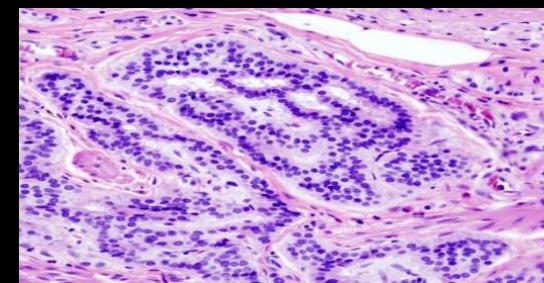
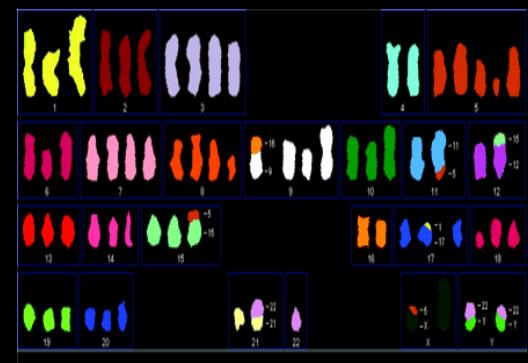
Chromosomal Instability



Genetic Aberrations

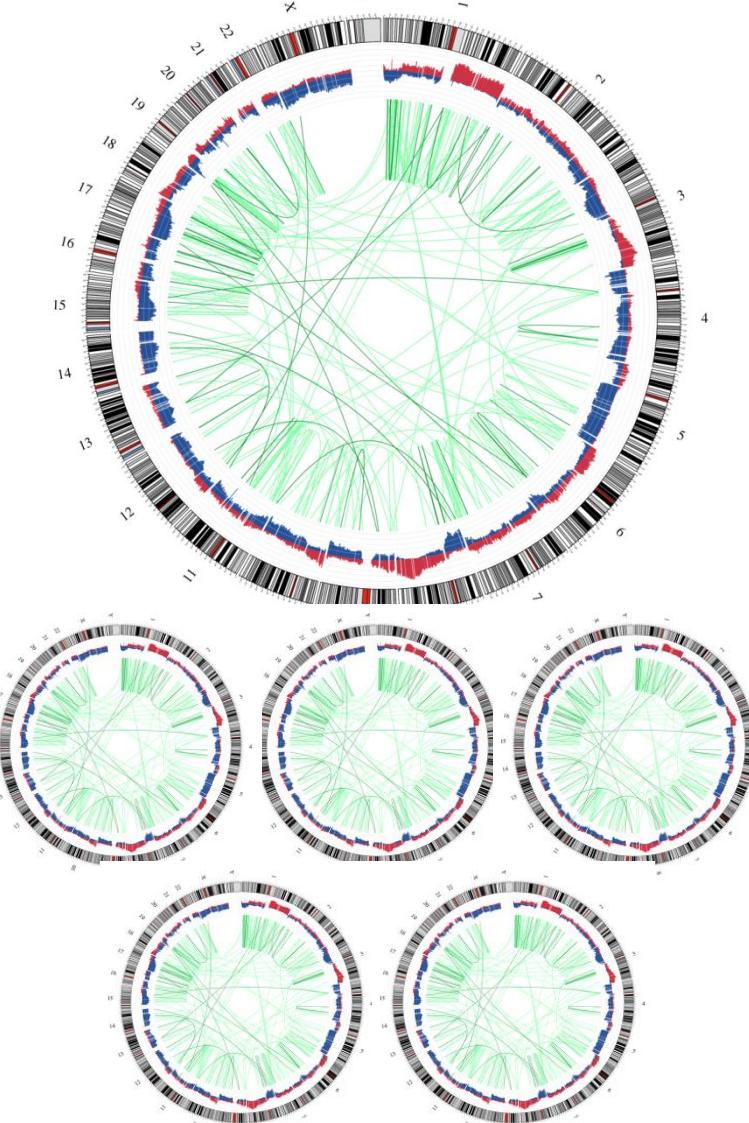


Malignancies

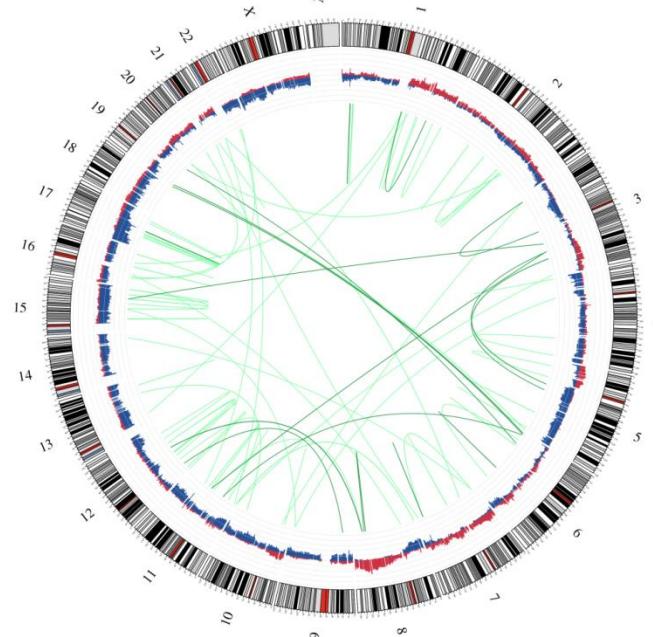


Basal cancers: much more re-arrangements

Basal 15%



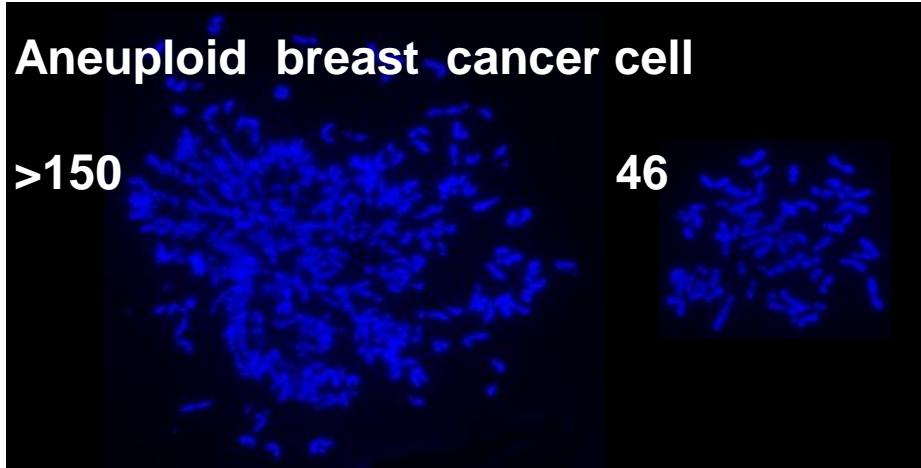
Non-basal 85%



Aneuploid breast cancer cell

>150

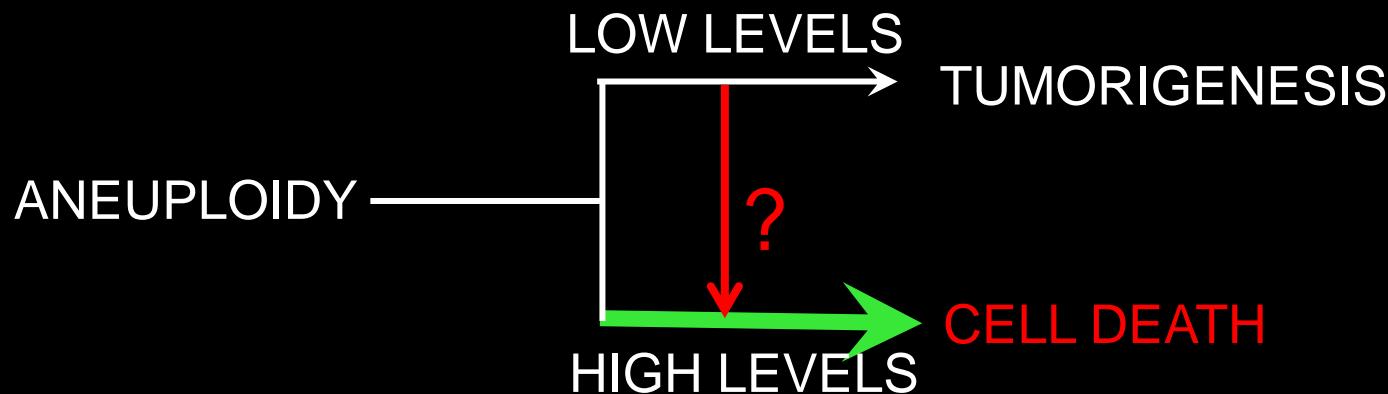
46



• triple Negative Breast Cancer mutations. Aparicio et al 2012

The Consequences of Aneuploidy

- Mouse models of mitotic checkpoint dysfunction have shown that aneuploidy can be causative for cancer
- Cancer is a potential outcome of aneuploidy (70%)
- Aneuploidy acts as tumor suppressor in certain contexts

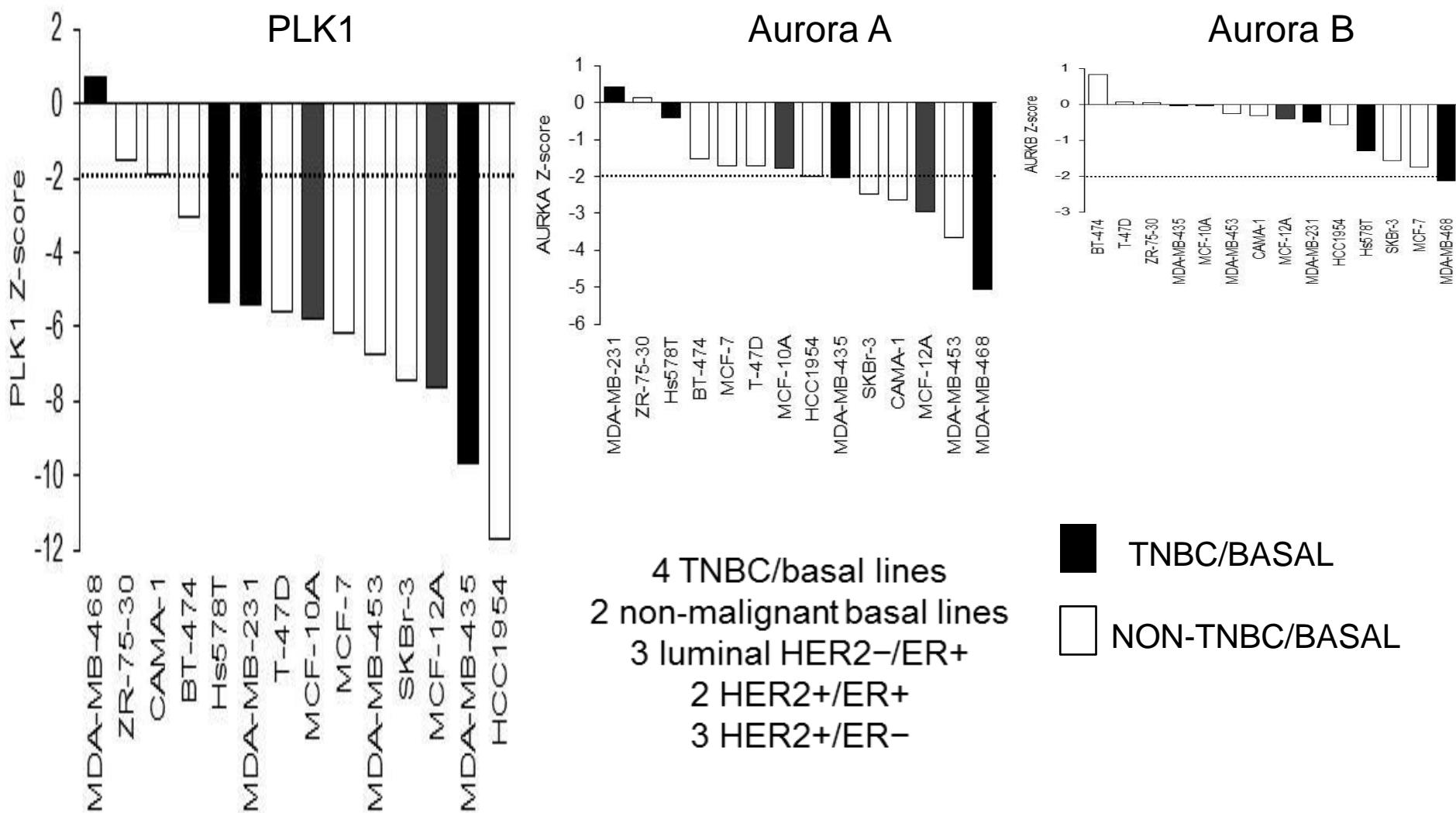


Whole kinome SiRNA screens on 14 breast cancer lines

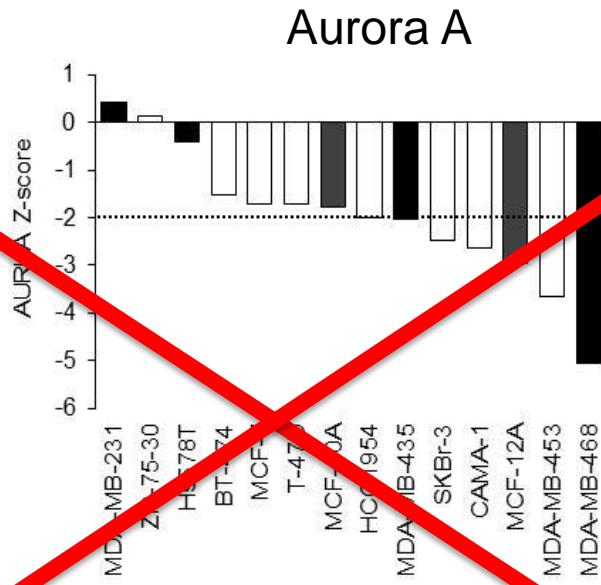
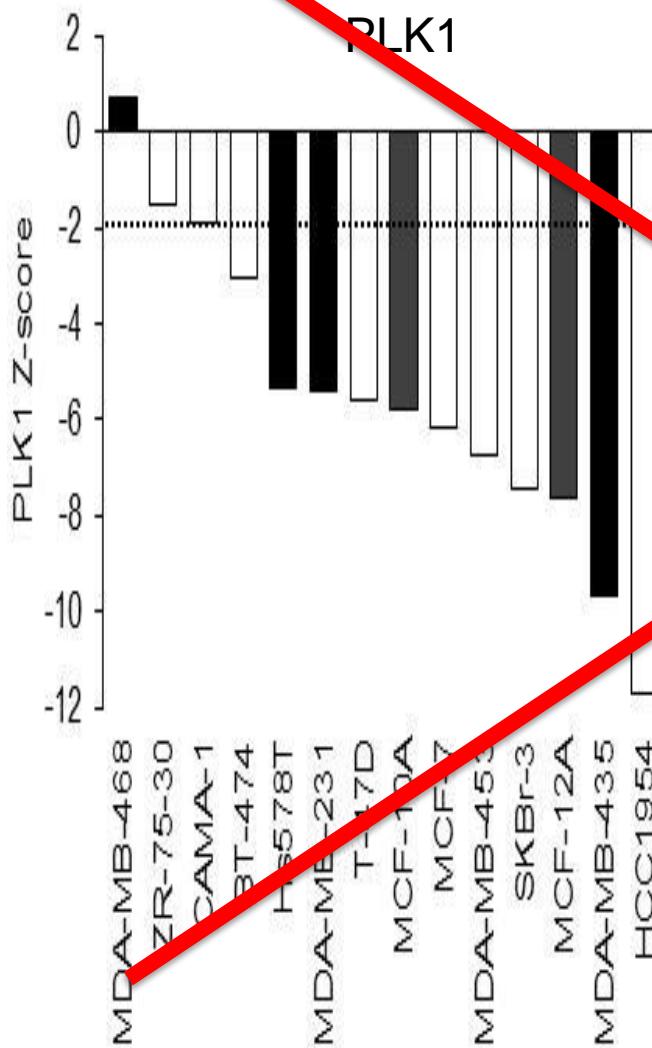


There is no silver bullet!
Limit your target to one cancer subtype!

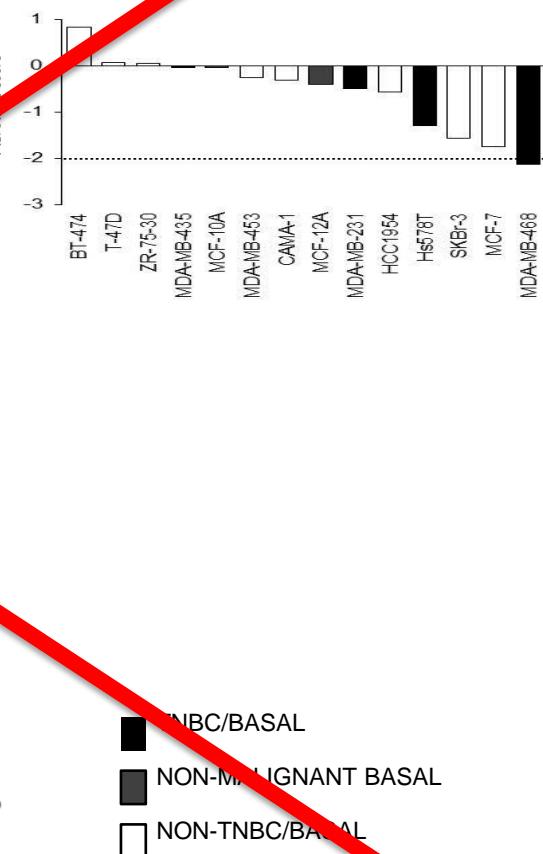
Kinome siRNA Z-scores of 14 Breast Cancer Cell Lines



Kinome siRNA Z-scores of 14 Breast Cancer Cell Lines

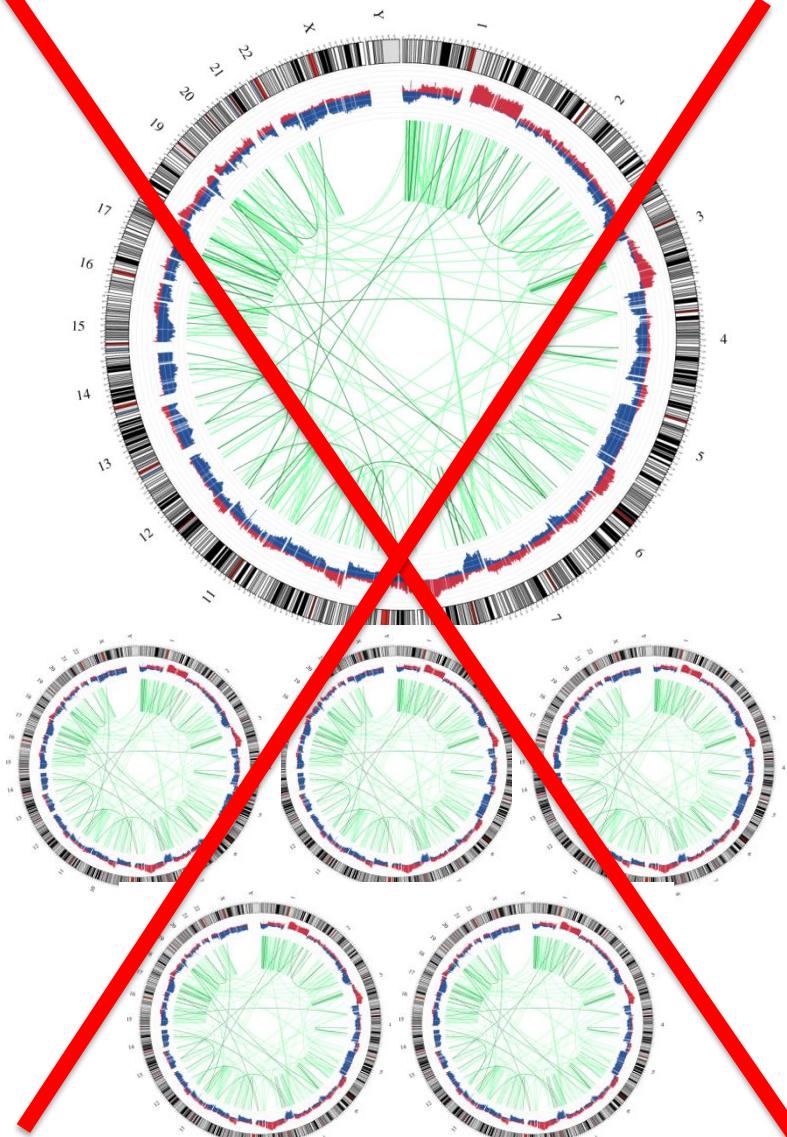


4 TNBC/basal lines
2 non-malignant basal lines
3 luminal HER2-/ER+
2 HER2+/ER+
3 HER2+/ER-

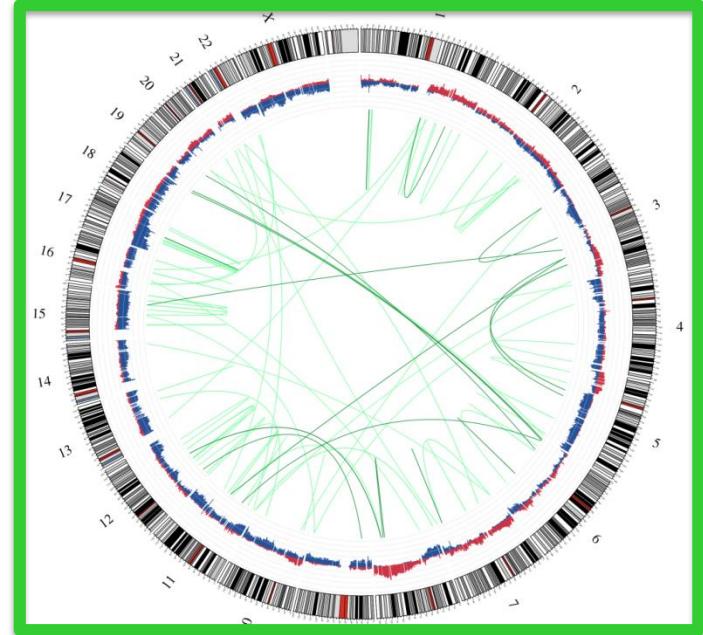


Basal cancers: much more re-arrangements

Basal 15%



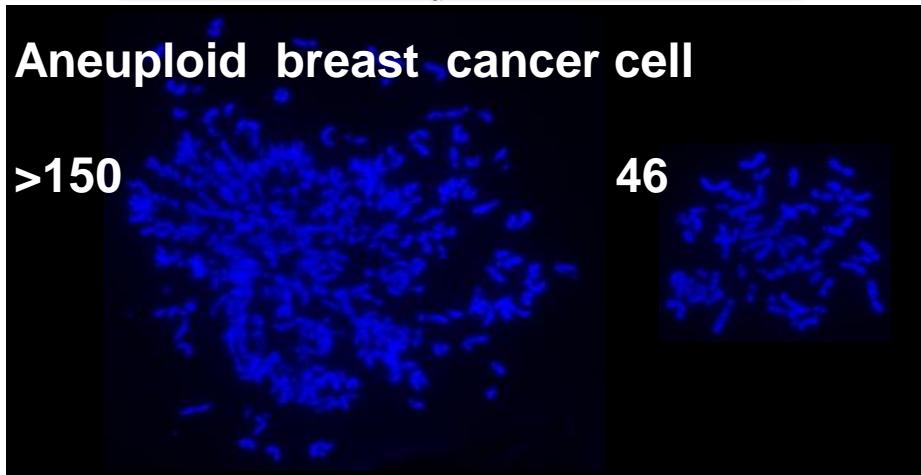
Non-basal 85%



Aneuploid breast cancer cell

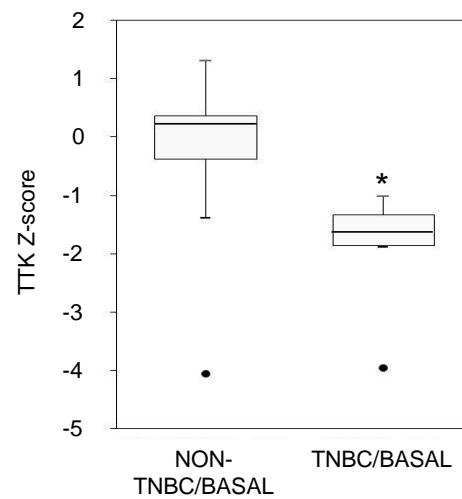
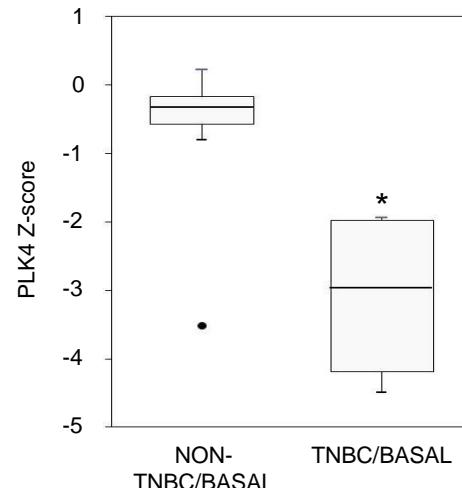
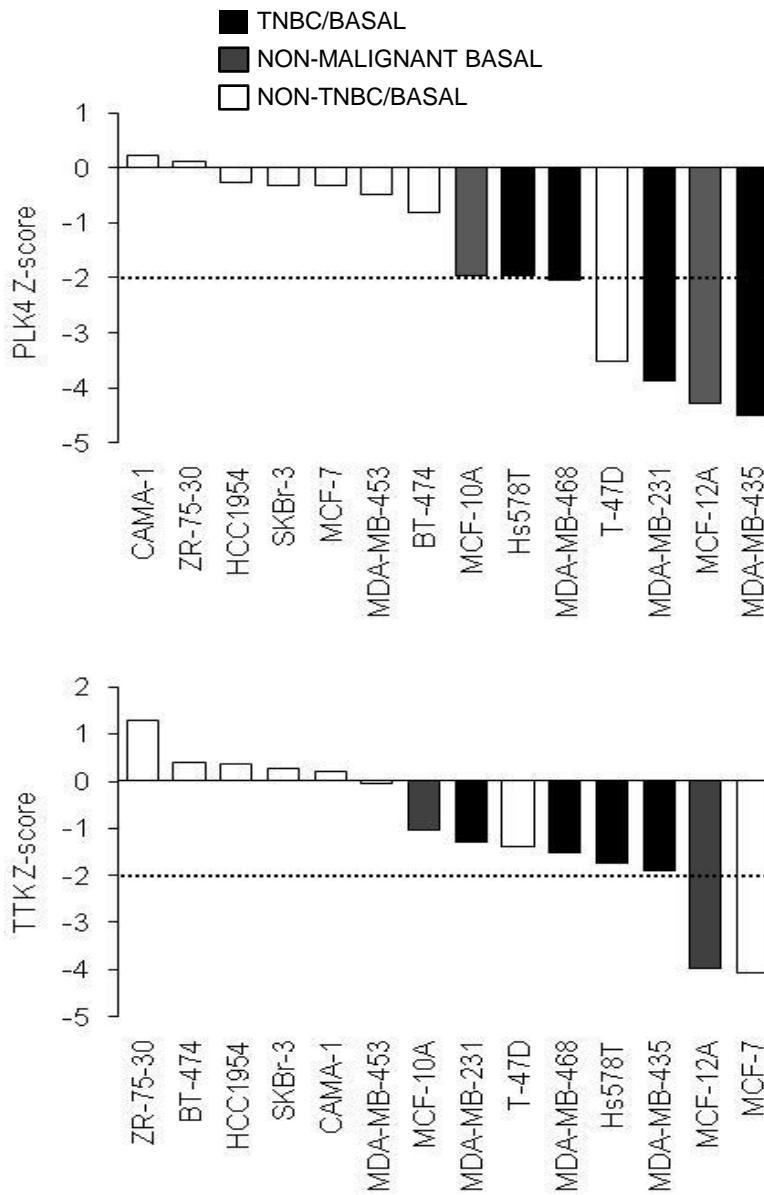
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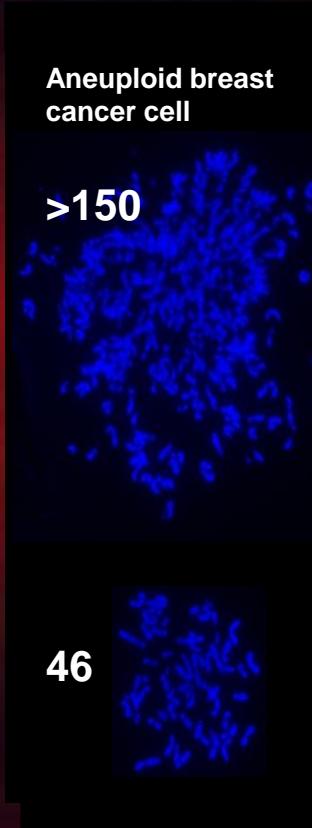
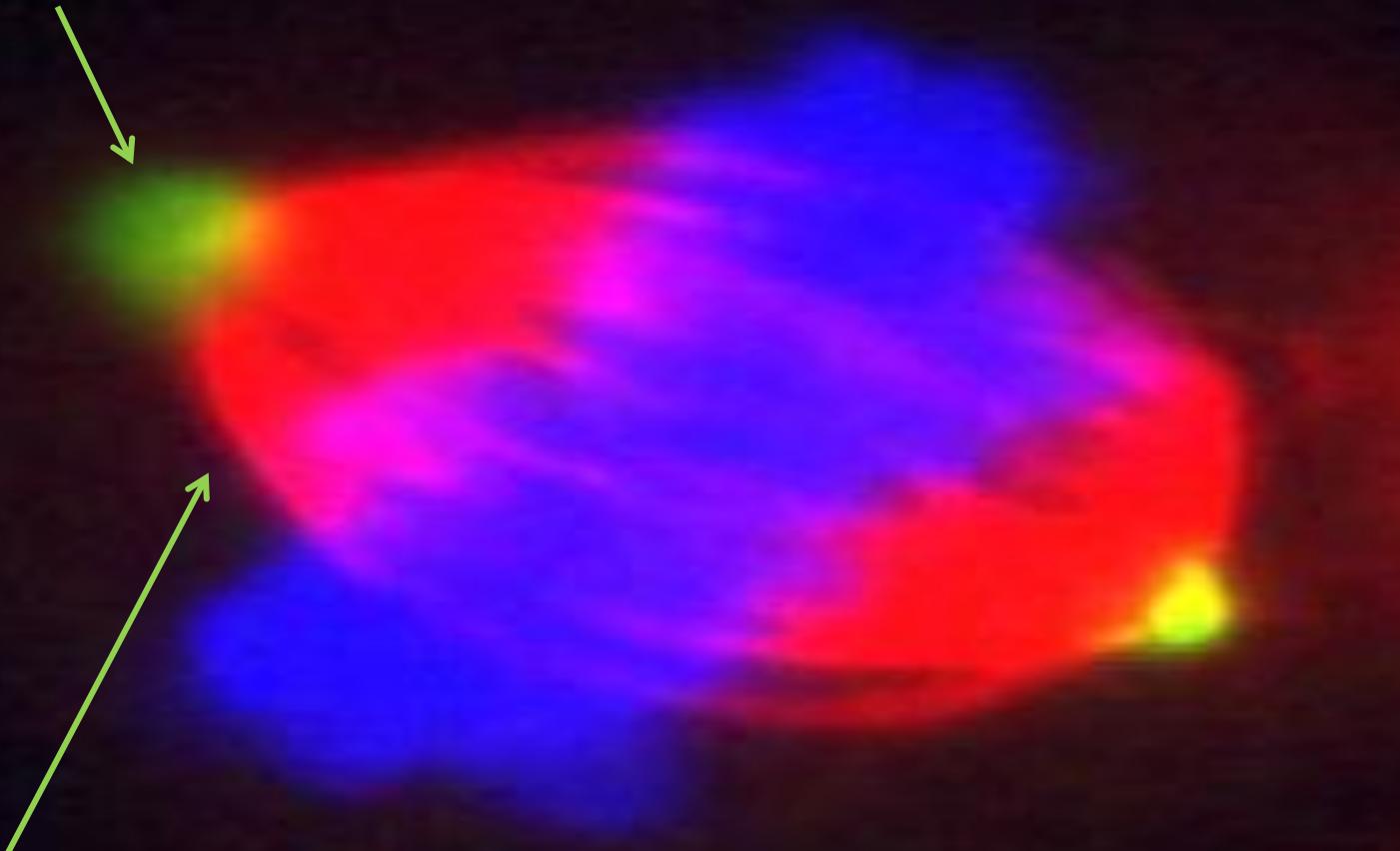
triple Negative Breast Cancer mutations. Aparicio et al 2012

PLK4 and TTK are Potential Therapeutic Targets for Triple-negative/Basal-like Breast Cancer



Aneuploidy

PLK4 centriole duplication CFI-400945



TTK spindle assembly checkpoint CFI1870

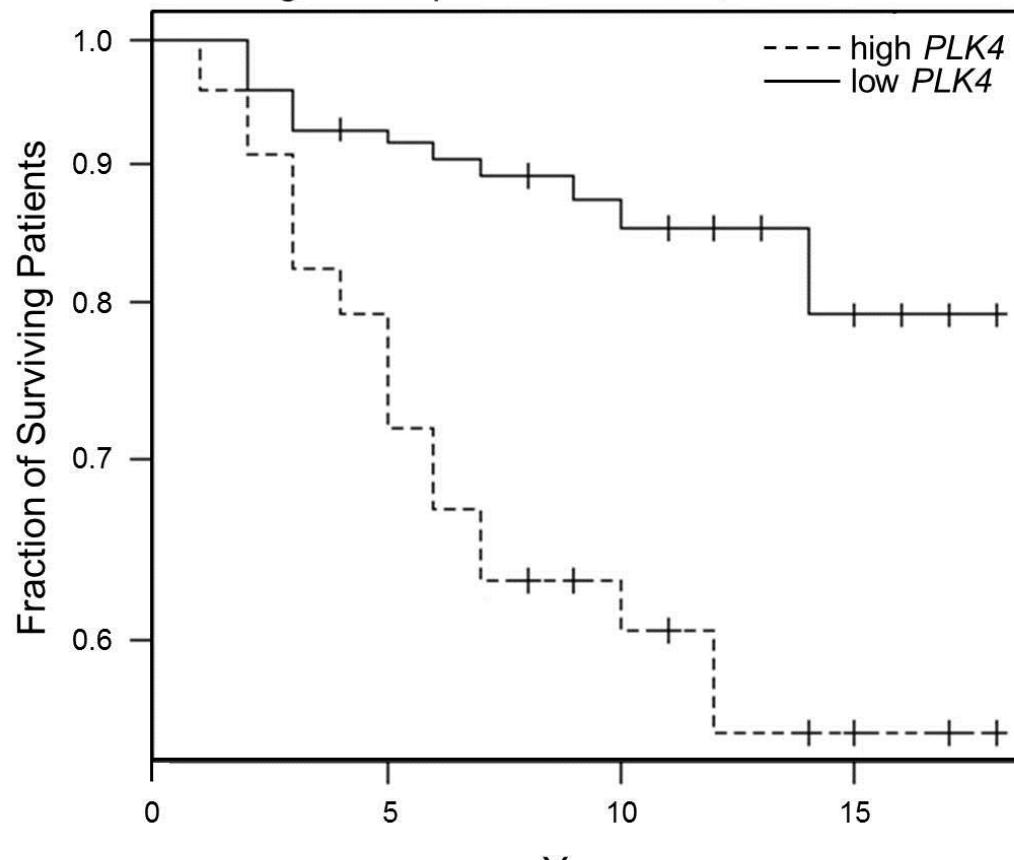
Expression of PLK4 in Breast Cancers and Prognosis

All Breast Cancers			Triple-negative/Basal-like			HER2+			Luminal A			Luminal B		
% Up	Ratio	p-value	% Up	Ratio	p-value	% Up	Ratio	p-value	% Up	Ratio	p-value	%Up	Ratio	p-value
26	1.46	8.5×10^{-4}	48	2.00	9.6×10^{-7}	19	1.38	0.021	3	1.06	0.65	35	1.69	0.0038

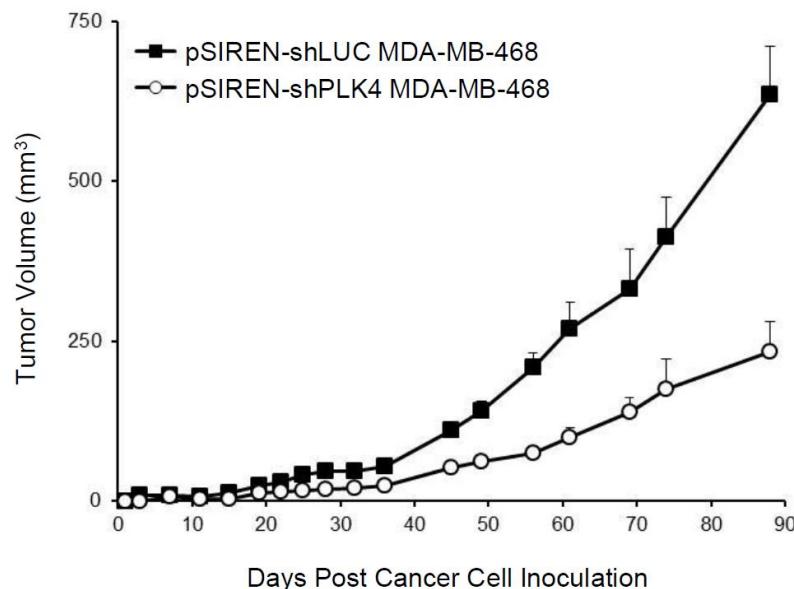
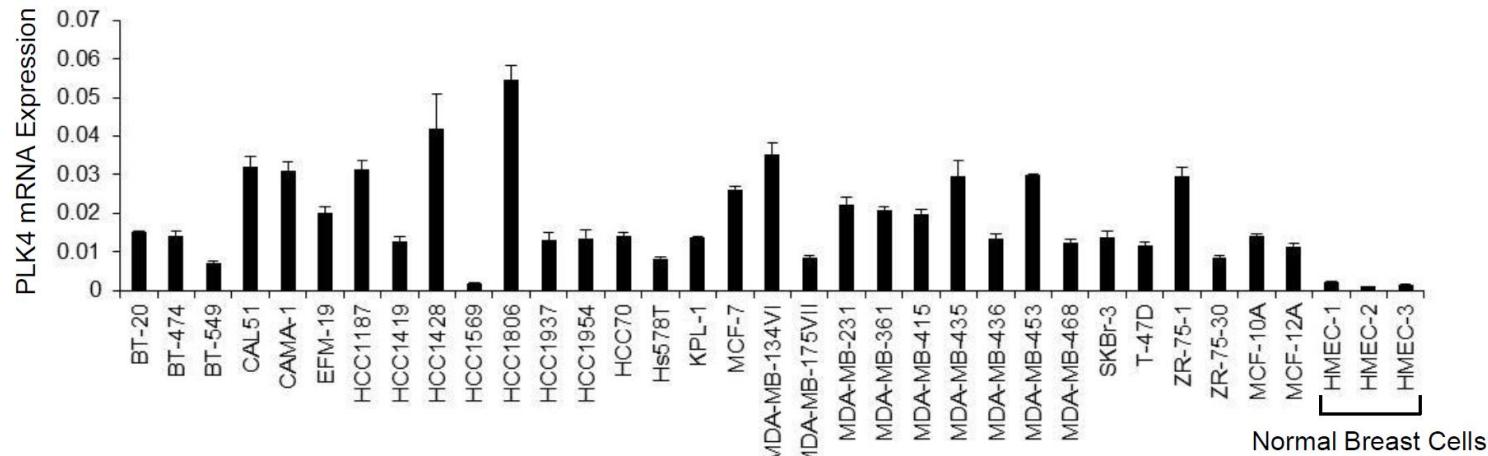
Hu et al., BMC Genomics; 7:96, 2006.

NEJM Dataset Survival vs. *PLK4*, probe NM_014264

Cox regression p-value = 7×10^{-5} , BH FDR = 0.002



Knockdown of PLK4 Inhibits Tumour Growth

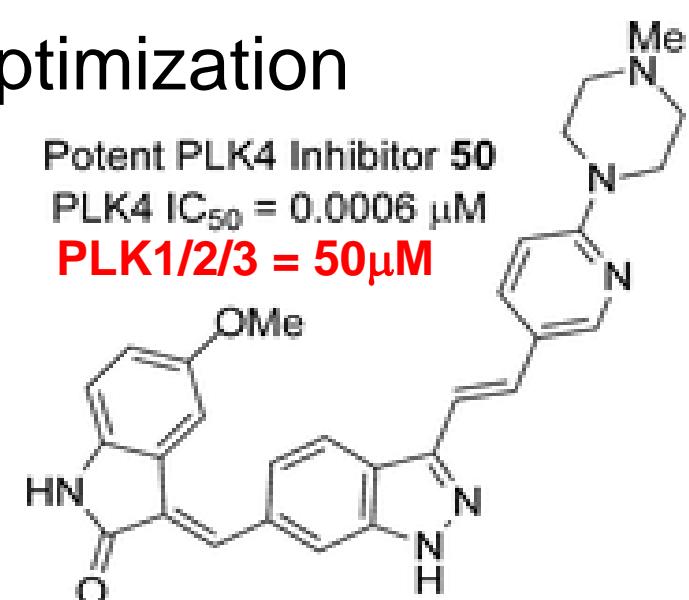
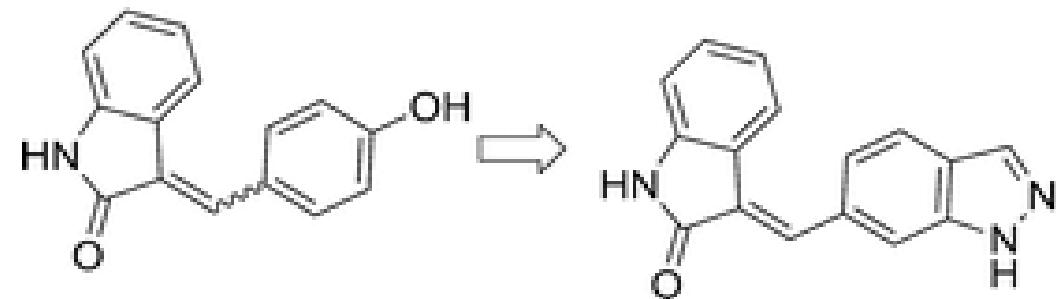


Structure-guided Lead Optimization

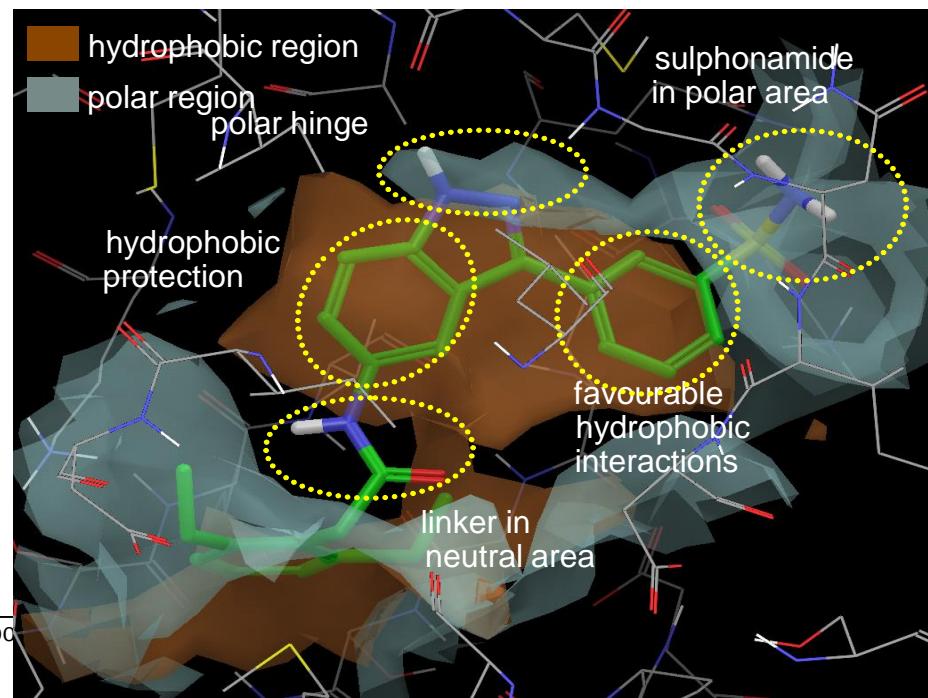
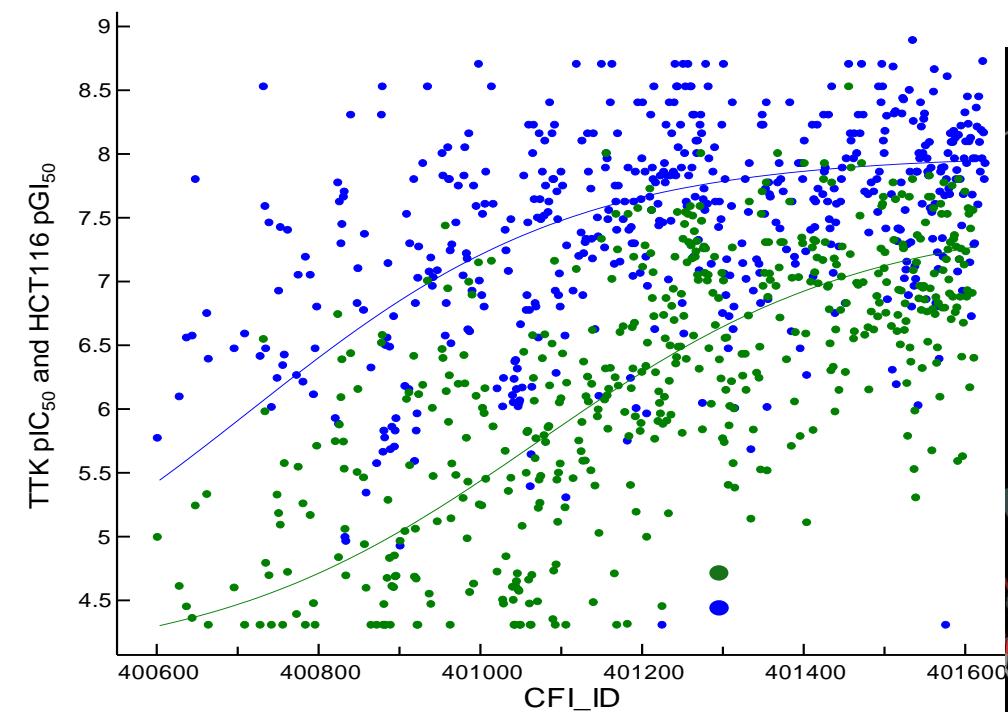
Screening Hit 11
PLK4 IC₅₀ = 32 μ M

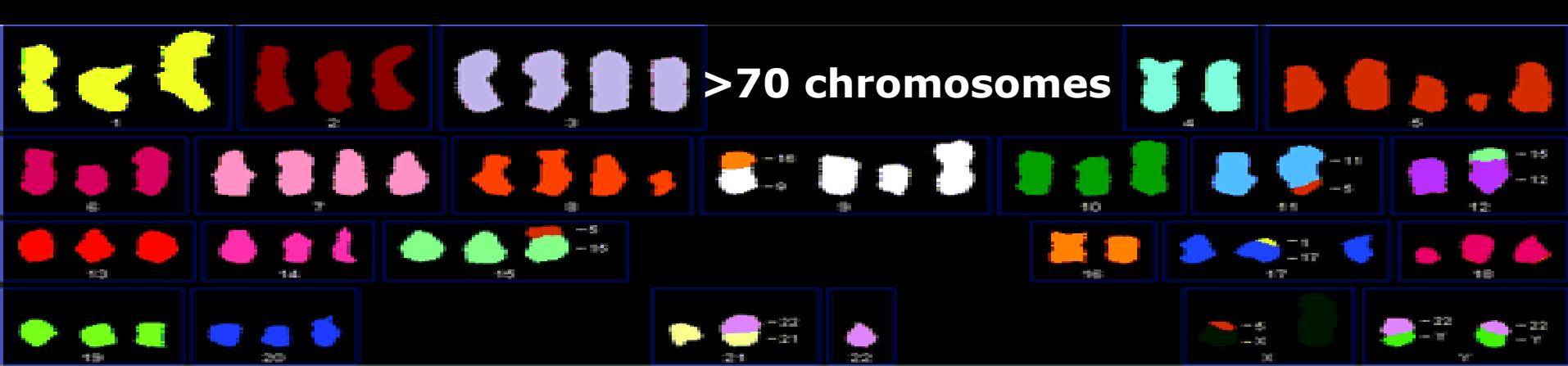
New Chemotype 17
PLK4 IC₅₀ = 0.29 μ M

Potent PLK4 Inhibitor 50
PLK4 IC₅₀ = 0.0006 μ M
PLK1/2/3 = 50 μ M



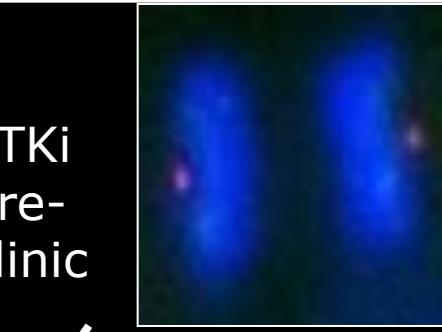
J. Med. Chem., 2013, 56:6069





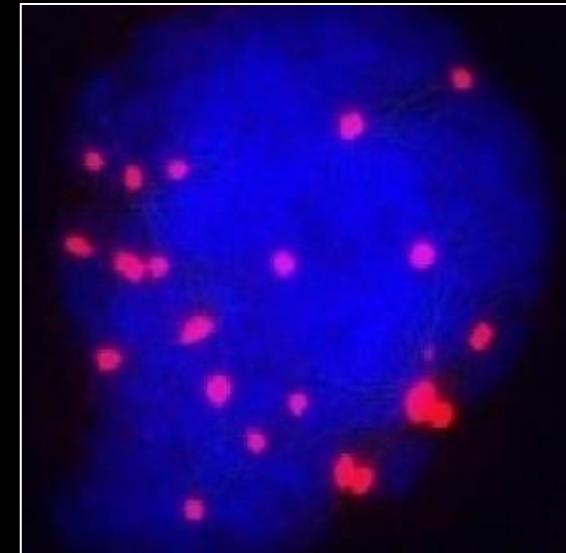
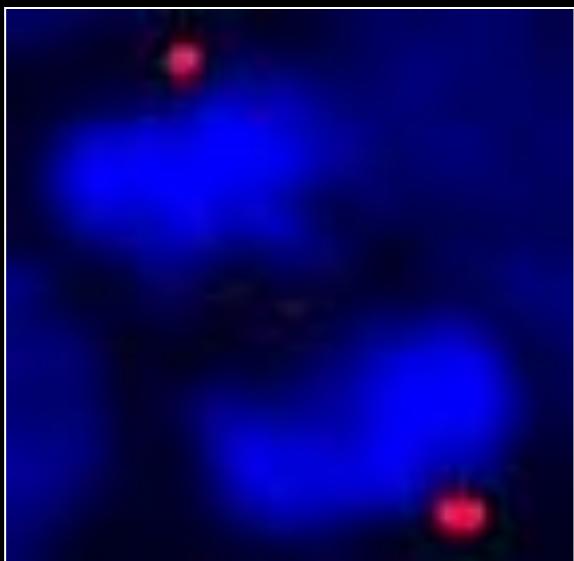
Jeremy Squires

TTKi
Pre-
clinic



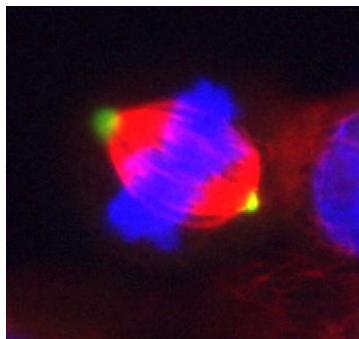
PLK4i
IND approved 11/13
1st patient dosed 4/14

1 compound 2



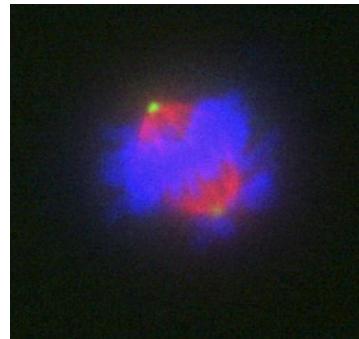
Contrasting Cellular Phenotypes Induced by Mitotic Kinase Inhibitors

DMSO



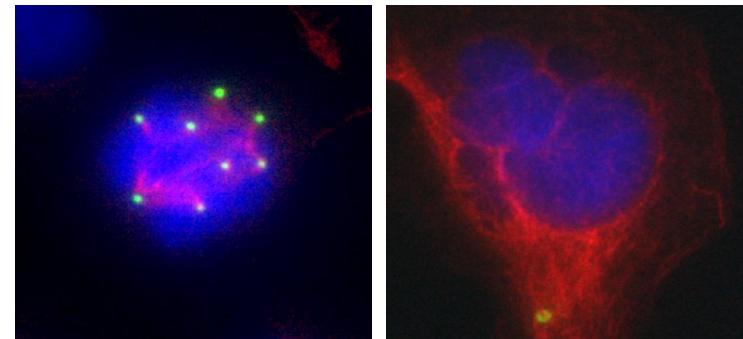
PERICENTRIN
ALPHA-TUBULIN
DAPI

CFI-TTK-001



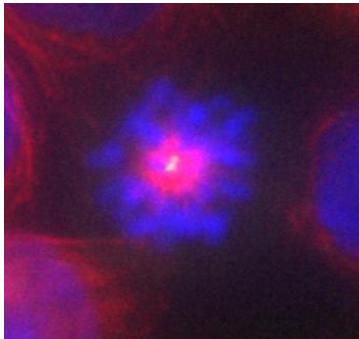
Segregation Errors
Mitotic Acceleration
Aneuploidy

CFI-PLK4-002



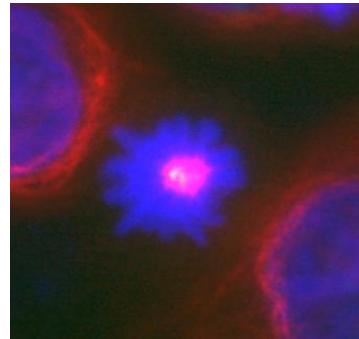
Centrosome Amplification/Multipolar Division
Failed Cytokinesis/Polypliody

BI2536 (PLK1i)



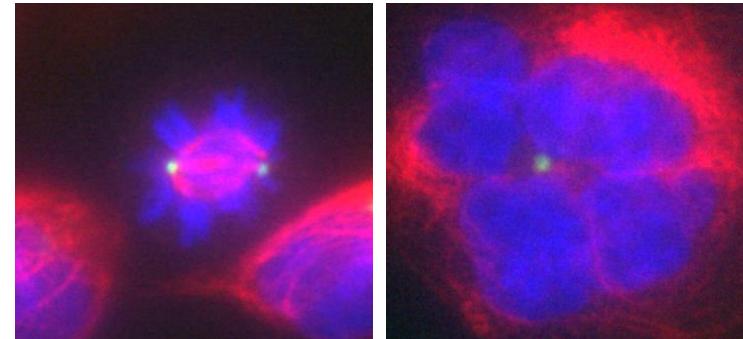
Monopolar Spindles
Mitotic Arrest/Death

MLN8054 (AURKAi)

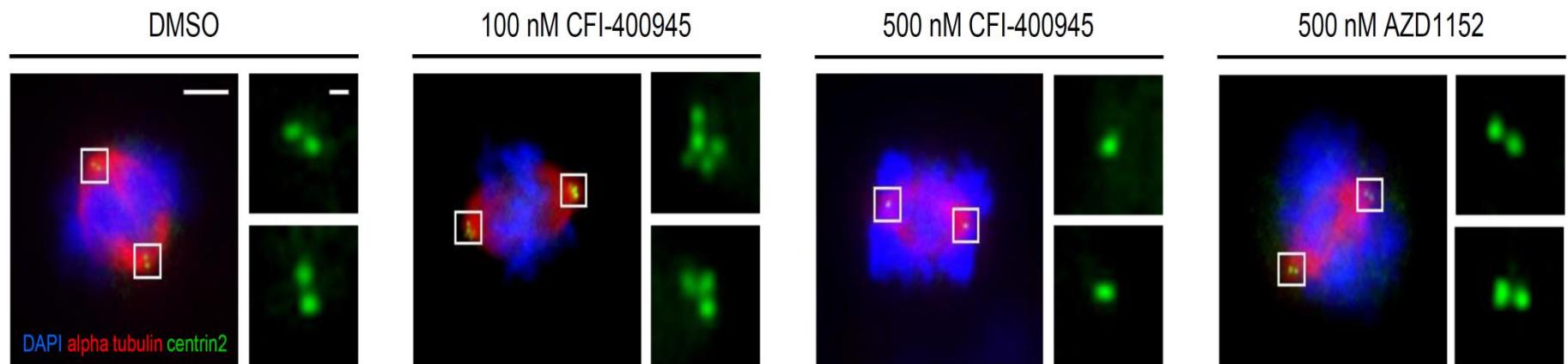


Monopolar Spindles
Mitotic Delay
Aneuploidy

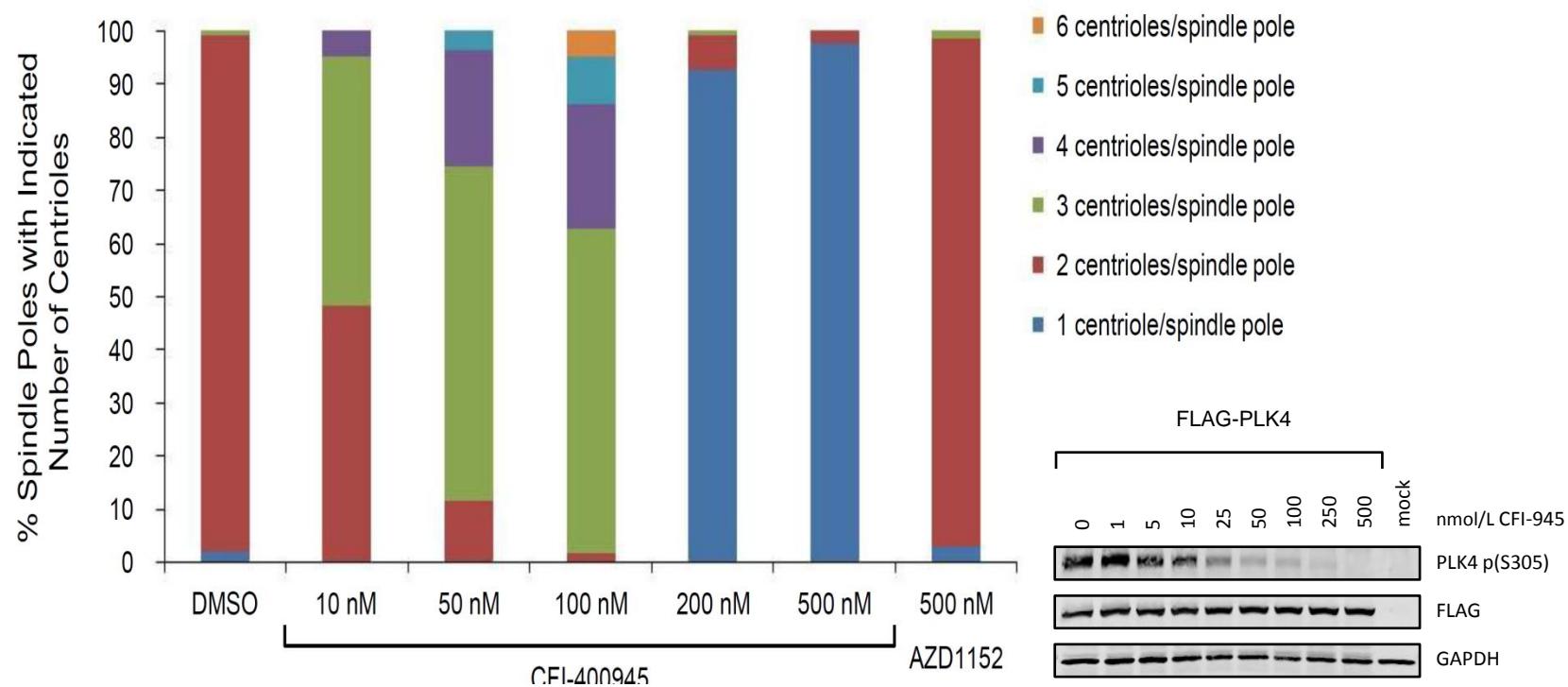
AZD1152 (AURKBi)



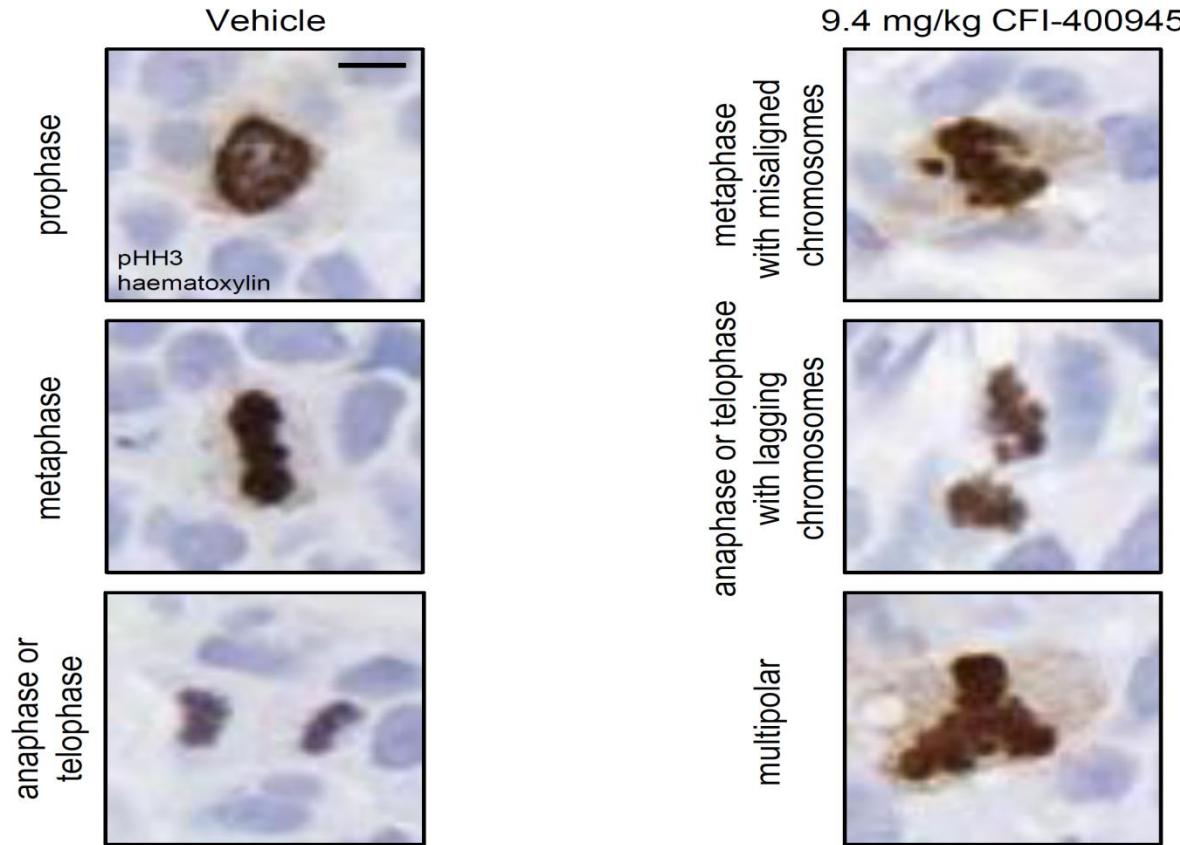
Misalignments
Failed Cytokinesis
Polypliody



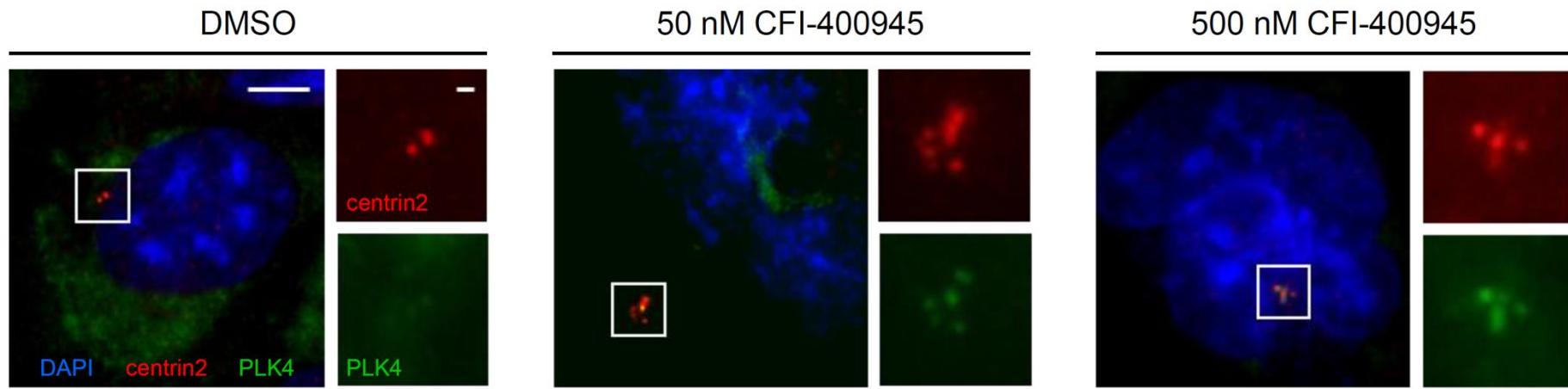
CFI-400945 Causes Centriole Duplication Defects in Cancer Cells



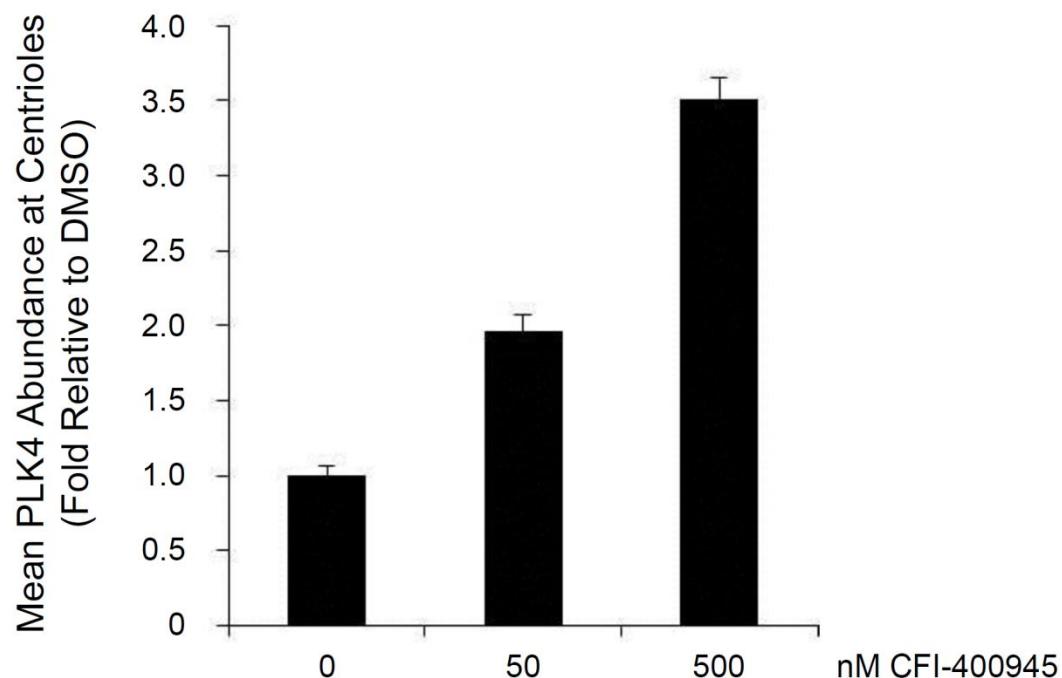
Effects of CFI-400945 on p-HH3 & aberrant mitosis in PDX xenografts.



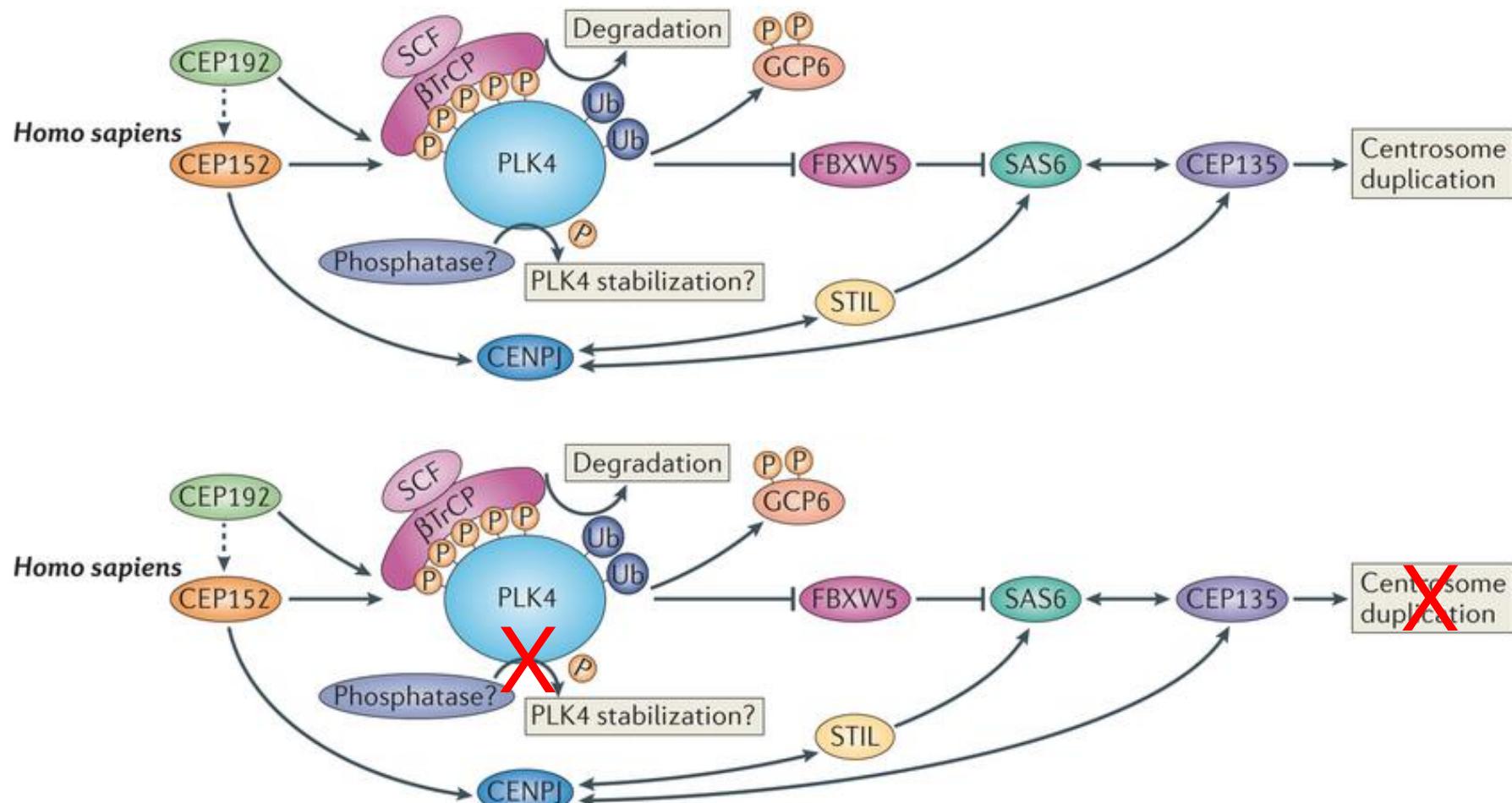
Test Article	Phospho-Histone H3 (Ser10) Positive Cells ¹	Aberrant Mitoses ²		
		Metaphase with Misaligned Chromosomes	Anaphase or Telophase with Lagging Chromosomes	Multipolar
Vehicle	$2.1 \pm 0.1 \%$	$1.3 \pm 0.5 \%$	$1.2 \pm 0.3 \%$	$1.0 \pm 0.3 \%$
9.4 mg/kg CFI-400945	$2.7 \pm 0.1 \%$	$5.0 \pm 1.2 \%$	$2.5 \pm 1.0 \%$	$4.1 \pm 0.4 \%$



CFI-400945 Increases the Level of PLK4 at Centrioles

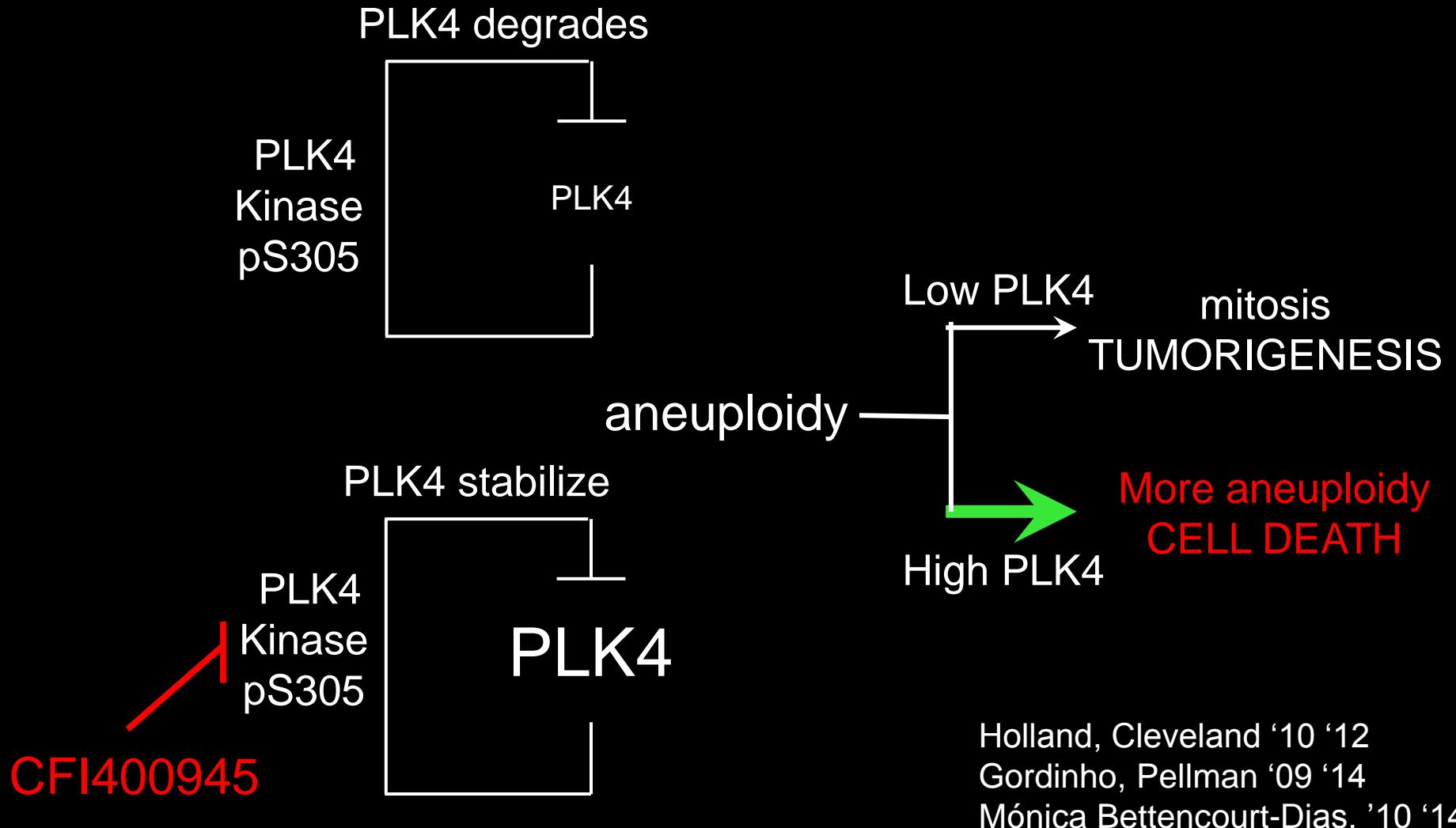


Centrosome Duplication Requires PLK4 Degradation



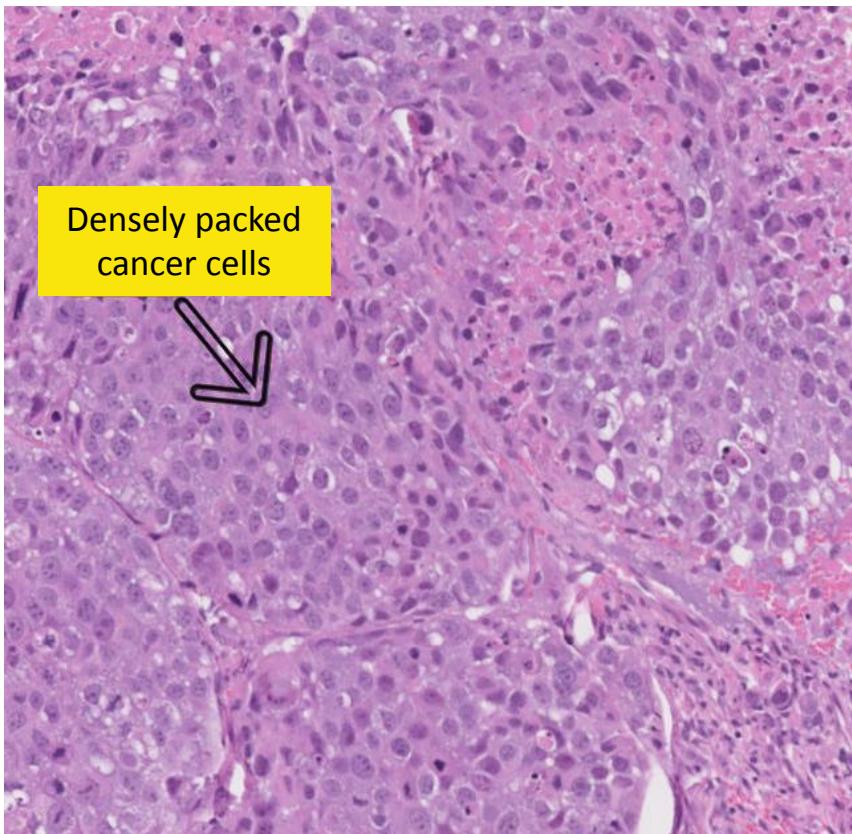
PLK4 auto-phosphorylation needed for degradation
Mónica Bettencourt-Dias '14 NRMCB

PKL4i converts tumorigenic initiation role of aneuploidy to inducing cell death

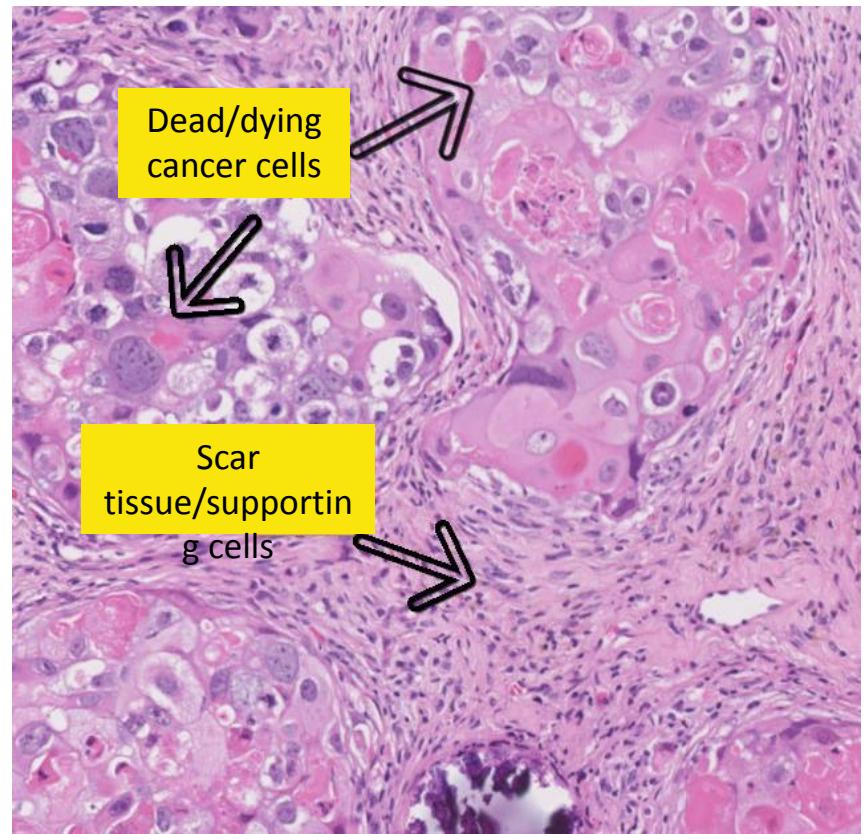


Profound cancer cell killing by CFI-400945

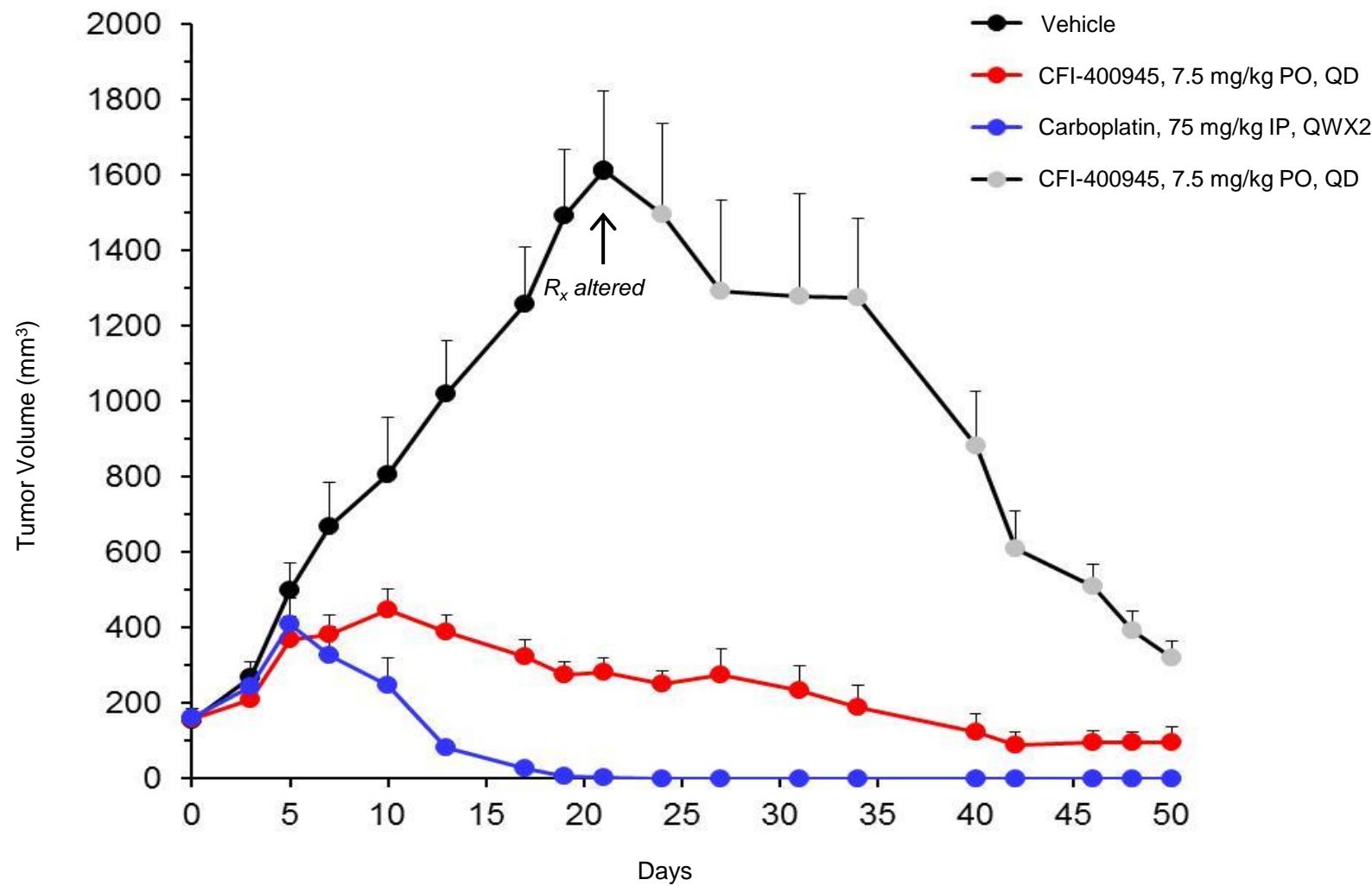
Untreated



Treated with CFI-400945



Antitumor Effects of CFI-400945 on a Carboplatin-sensitive Ovarian PDX



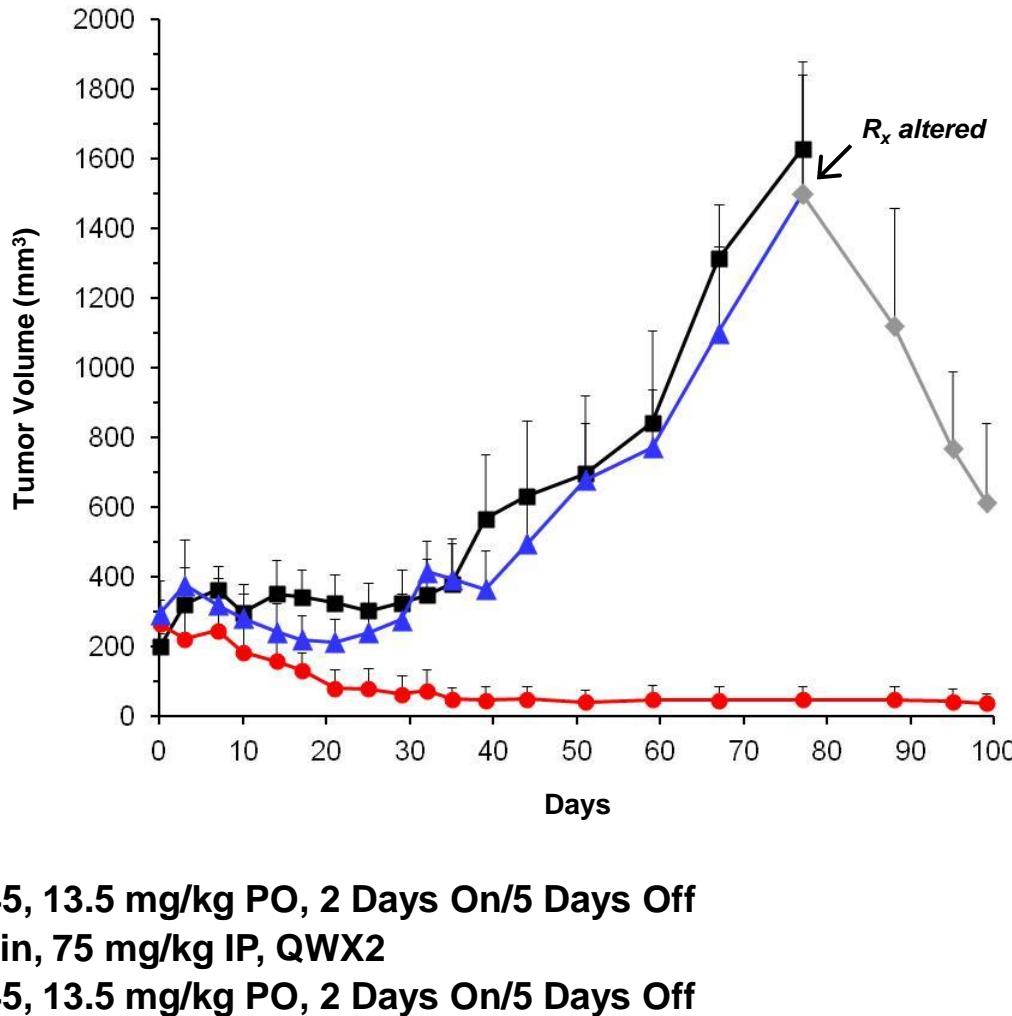
CDDP Resistance Does Not Affect Sensitivity of Ovarian Cancer Cells to CFI-400945

CELL LINE	GROWTH INHIBITION, GI ₅₀ (μM)	
	CFI-400945	CDDP
OVCAR-5	0.01	0.85
OVCAR-5-CisR	0.007	12
SKOV-3	0.01	0.66
SKOV-3-cis	0.007	3.7
A2780	0.007	0.58
A2780/CP70	0.009	13

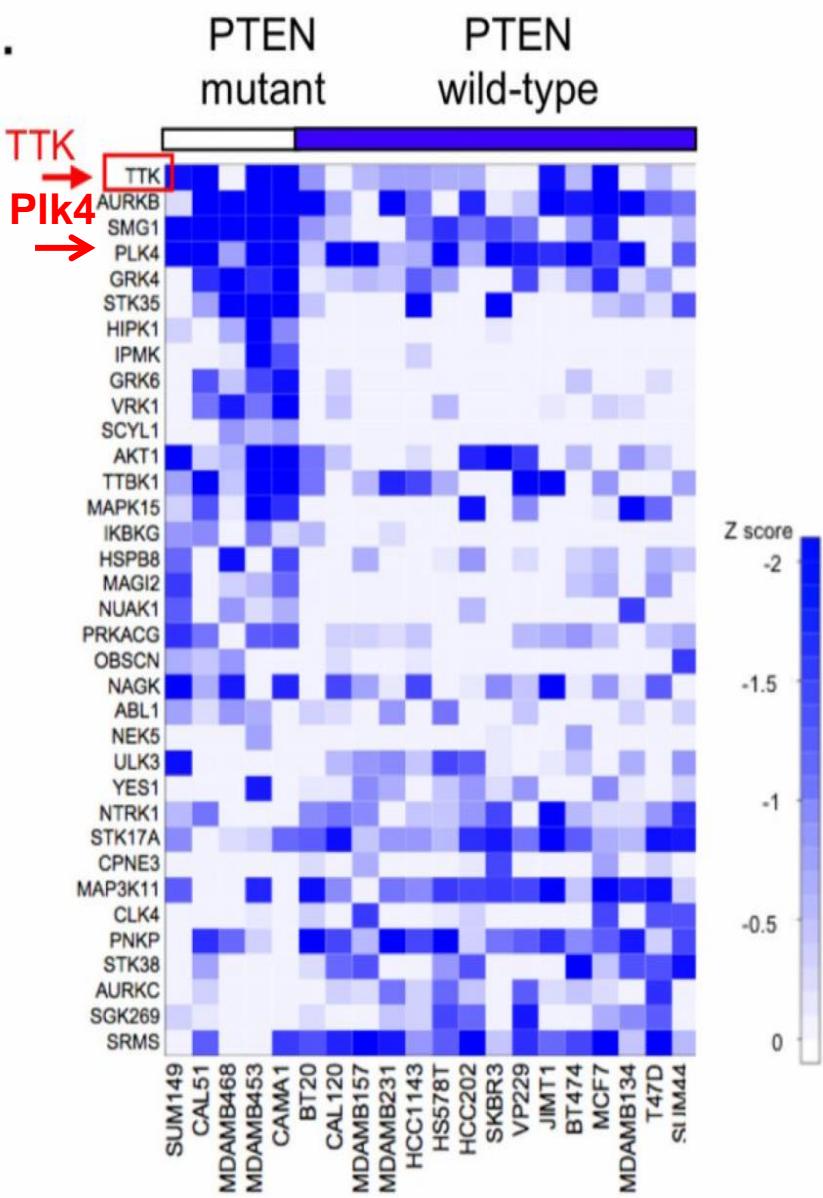
Cells were treated with CFI-400945 for 5 days, and GI₅₀s were measured by cell counts, except for A2780 and A2780/CP70 cells which were measured by Alamar Blue assay.

CFI-400945 in a Breast Cancer PDX

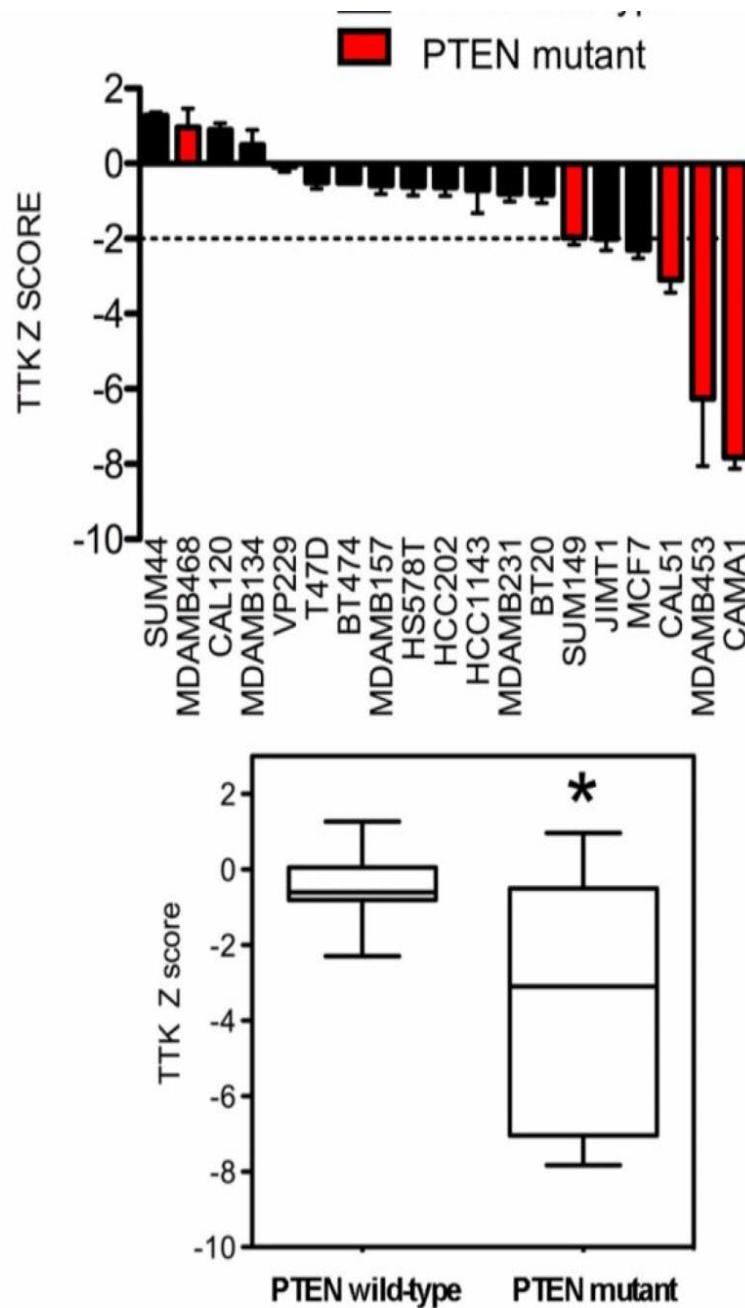
Primary Human Breast Cancer (ER^{low}/PR-/HER2-, PTEN Null)



A.



B.

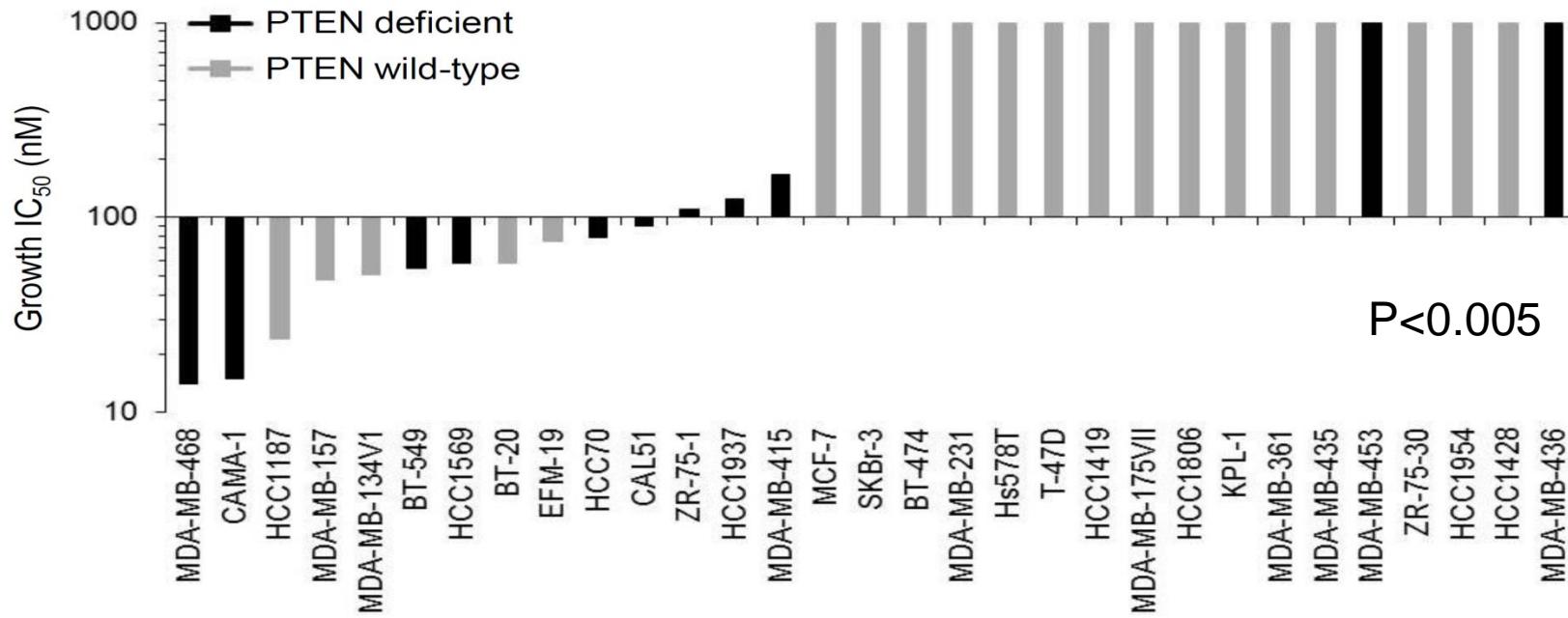


Percent Involvement of PTEN, BRCA1, p53 Breast Ca Subtypes

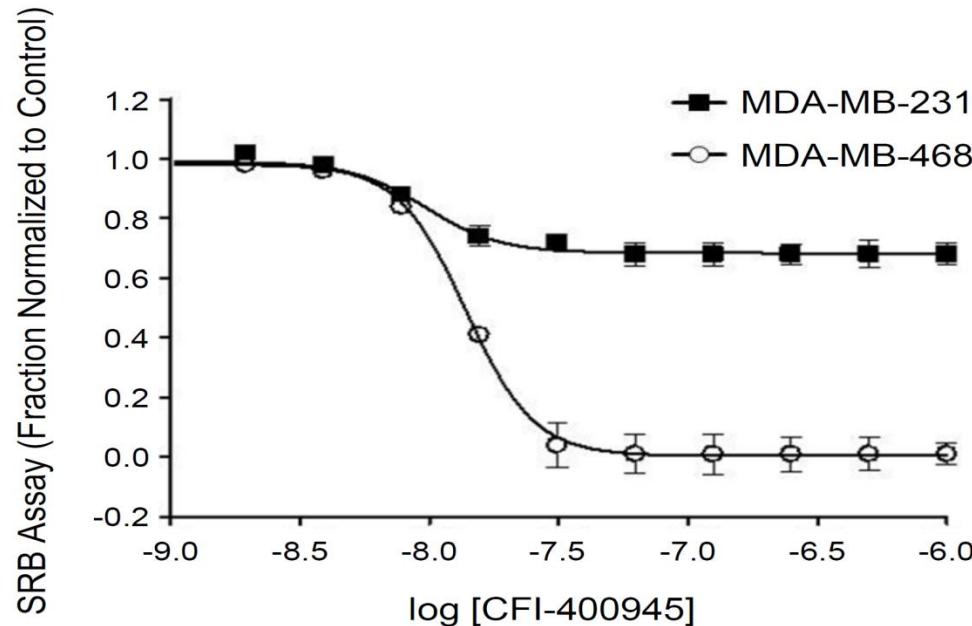
	LumA	LumB	Her-2	Basal	Ovarian
PTEN *	15	27	19	42	40
BRCA1	3	2	3	24	18
P53	12	30	72	77	96
At least 1	27	47	78	91	96
5 years survival	90	70	50	40	20

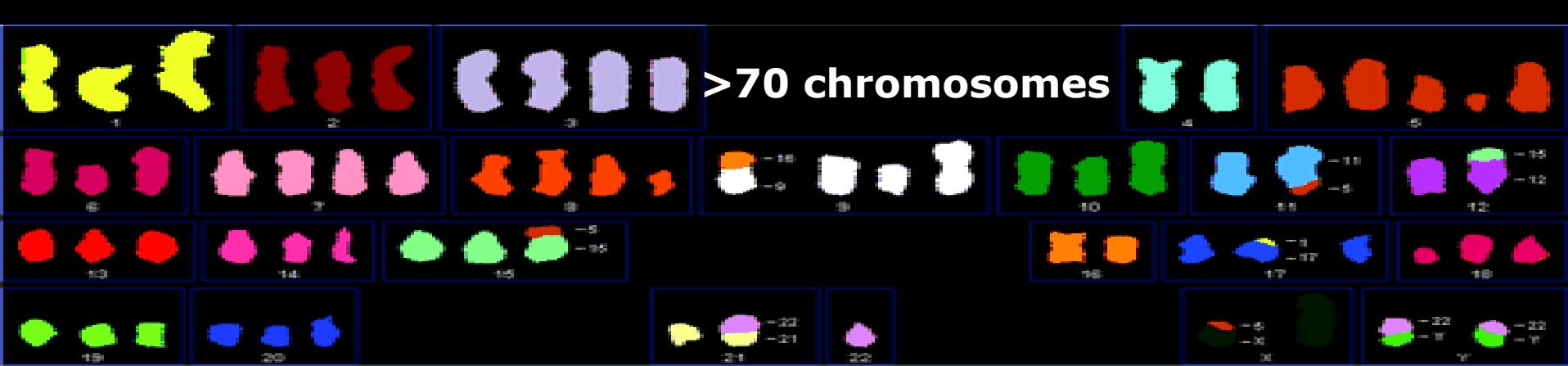
Nature. 2012 Sep 23. doi: 10.1038/nature11412.

*Nuclear PTEN controls homologous recombination mediated DNA repair and sensitivity to DNA damage Bassi et al Science 2013



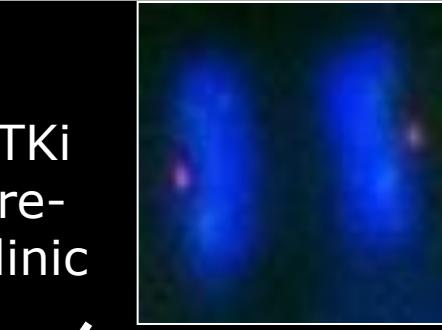
Response Profile of CFI-400945 Versus Breast Cancer Cell Lines.





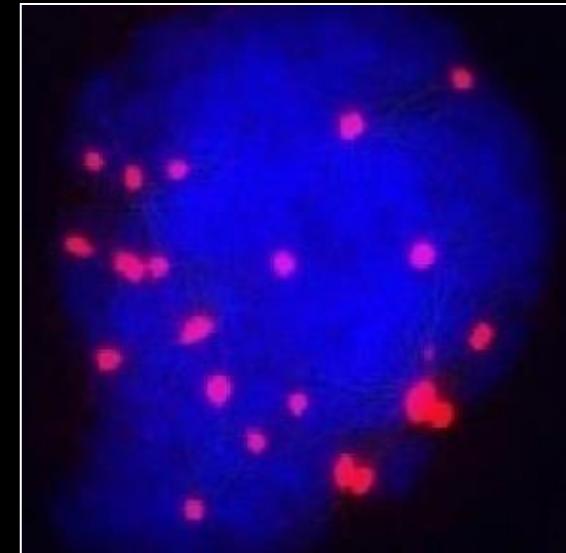
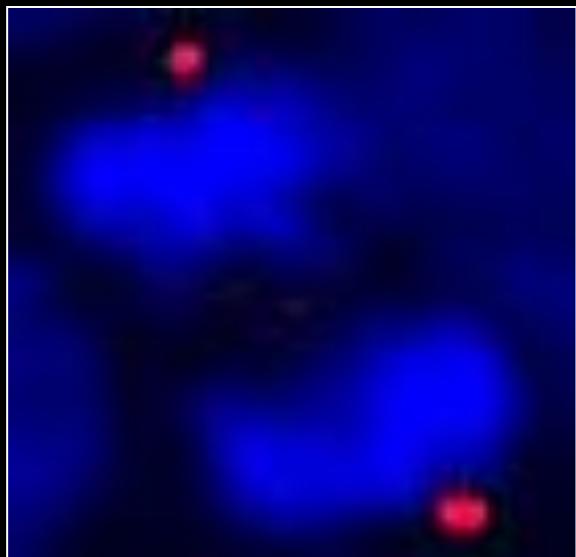
Jeremy Squires

TTKi
Pre-
clinic



PLK4i
IND approved 11/13
1st patient dosed 4/14

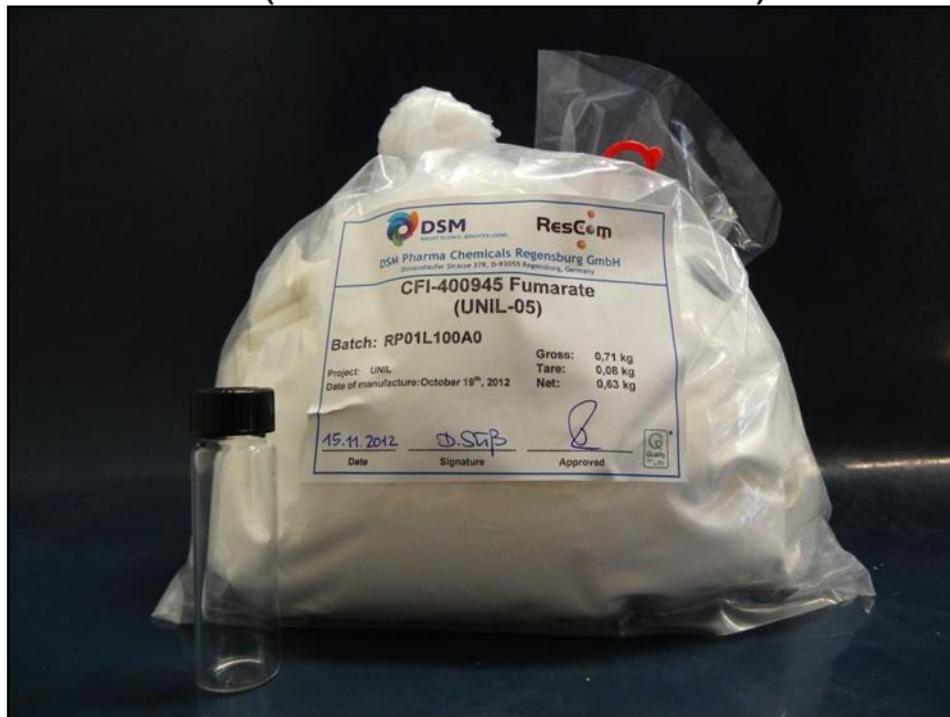
1 compound 2



CFI-400945

Drug Substance

(DSM Pharmaceuticals)



Drug Product

(Pharmatek)



670g GMP CFI-400945 fumarate produced
Sites opened :PMCC, Toronto
UCLA

290g API formulated
• 17,300 1.5 mg tablets
• 22,700 8 mg tablets



**Kathrin Zaugg Murray Robinson Rusty Jones
Masato Sasaki Christiane Knobbe Rob Cairns Stephan Gross Maria Figueroa Ari Melnick
Evan Lind Pam Ohashi Francois Lemonnier Philippe Gaulard
Chiara Gorrini Mona Gauthier
Mark Bray Jackie Mason Henry Pauls David Cescon David Hedley**