

Functional Profiling for “Targeted” Drug Therapy with Cell Culture Assays

Larry Weisenthal <http://weisenthal.org>

What is the best endpoint?

- Whole body function
- Whole tumor function
- Tumor cell function
- Protein activity
- Protein content
- RNA expression
- DNA content



Clinical
Relevance

Topics for Discussion

- Two endpoints for functional profiling (cell culture) assays: cell growth and **cell death**
- **Cell death assays (CDAs)** measure the same basic endpoint and the literature may be meta-analyzed.
- **CDAs** predict for individual outcomes (response and survival)

Cell Death Assays (CDAs), continued

- CDAs show disease-specific drug activity
- CDAs are useful clinical and research tools for “targeted” drugs, examples: Gefitinib and Bevacizumab
- CDAs provide unique information complementary to that provided by “molecular” tests

Cell culture assay endpoints

- Cell Proliferation
- Cell Death

Patient death

- Cessation of breathing
- Cessation of heart contractions
- Cessation of brain function
- Loss of body heat
- Rigor mortis
- Decomposition

All valid measurements of patient death

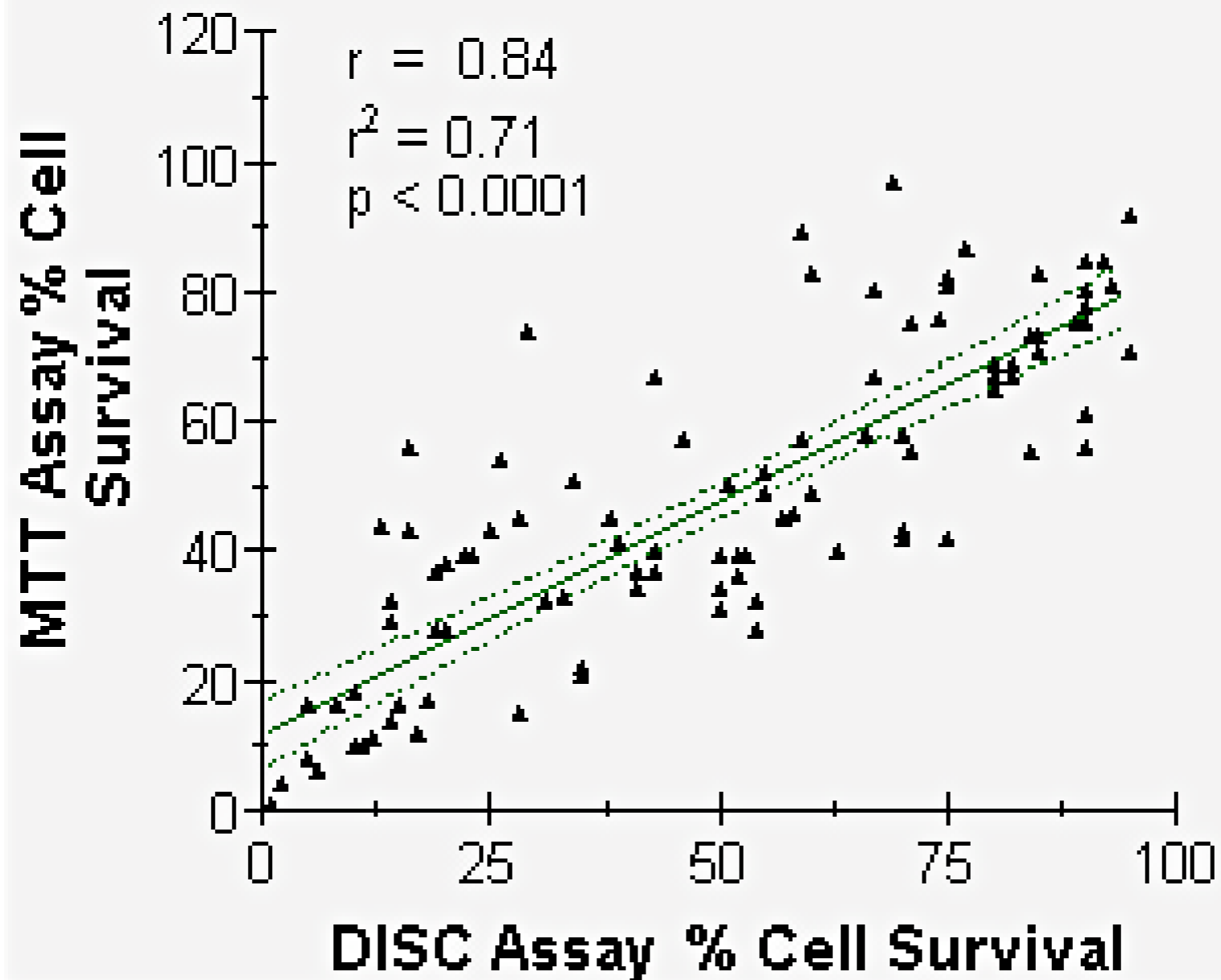
Cell death

- Membrane blebbing/exteriorization (MiCK; Annexin V FITC)
- Caspase activation
- DNA fragmentation (TUNEL)
- Membrane leakage (DISC/Fluorescein Diacetate)
- Mitochondrial (MTT)/Cellular (ATP/Resazurin) metabolic cessation.

All valid measurements of cell death

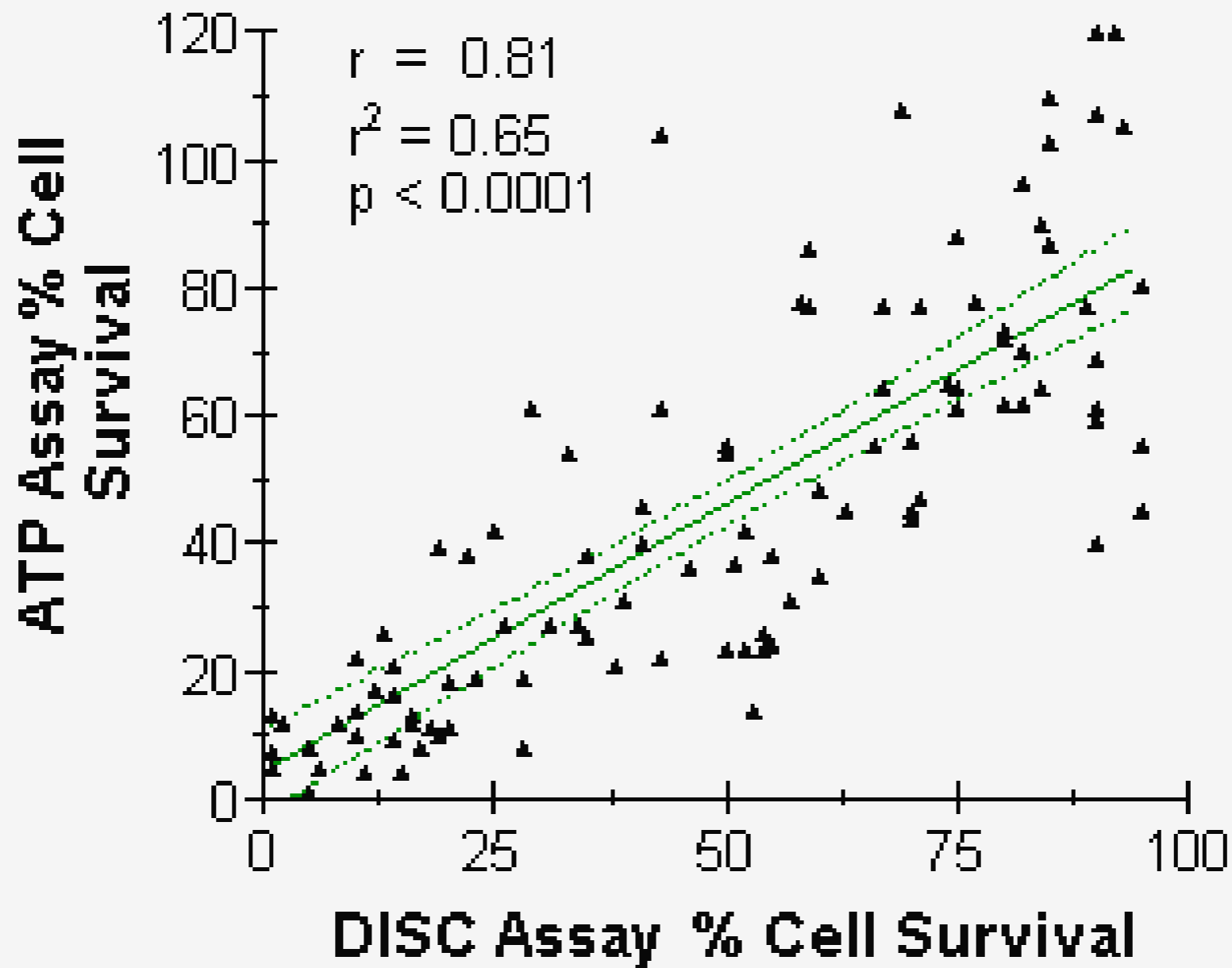
**Comparison between DISC and MTT assays; 20 drugs tested;
5 adenocarcinomas**

In green is regression line +/- 95% CI



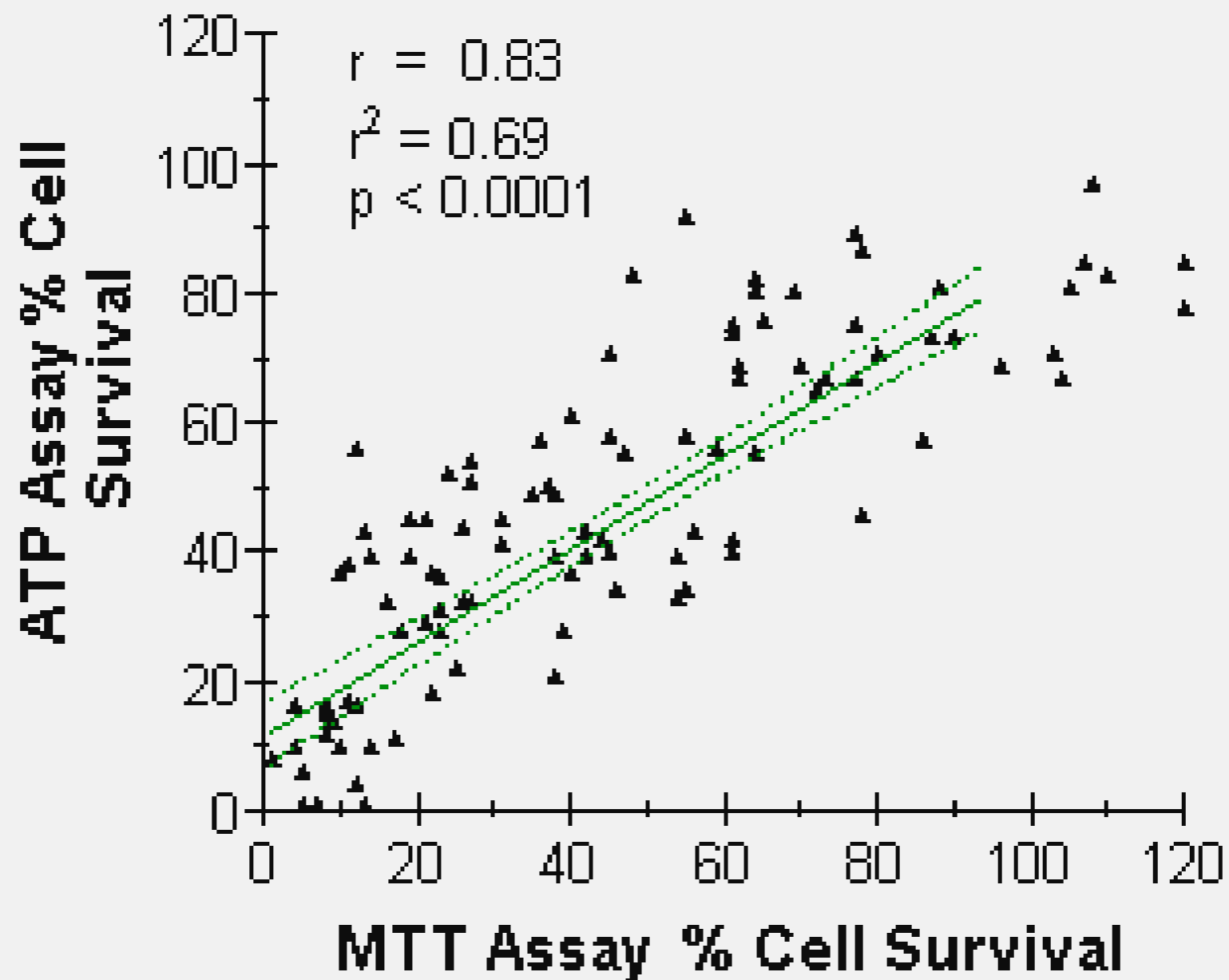
**Comparison between DISC and
ATP assays; 20 drugs tested;
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In green is regression line +/- 95% CI

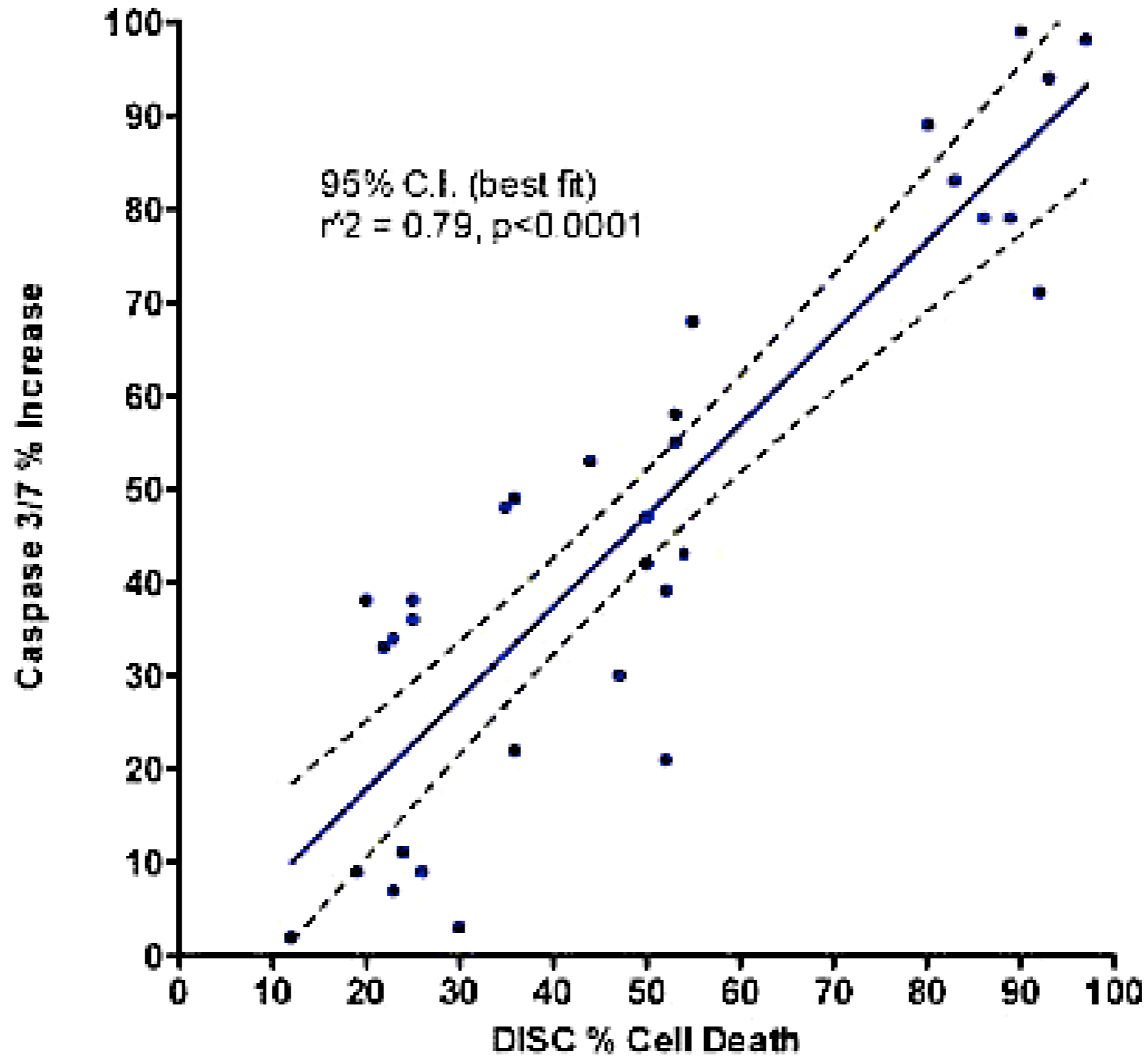


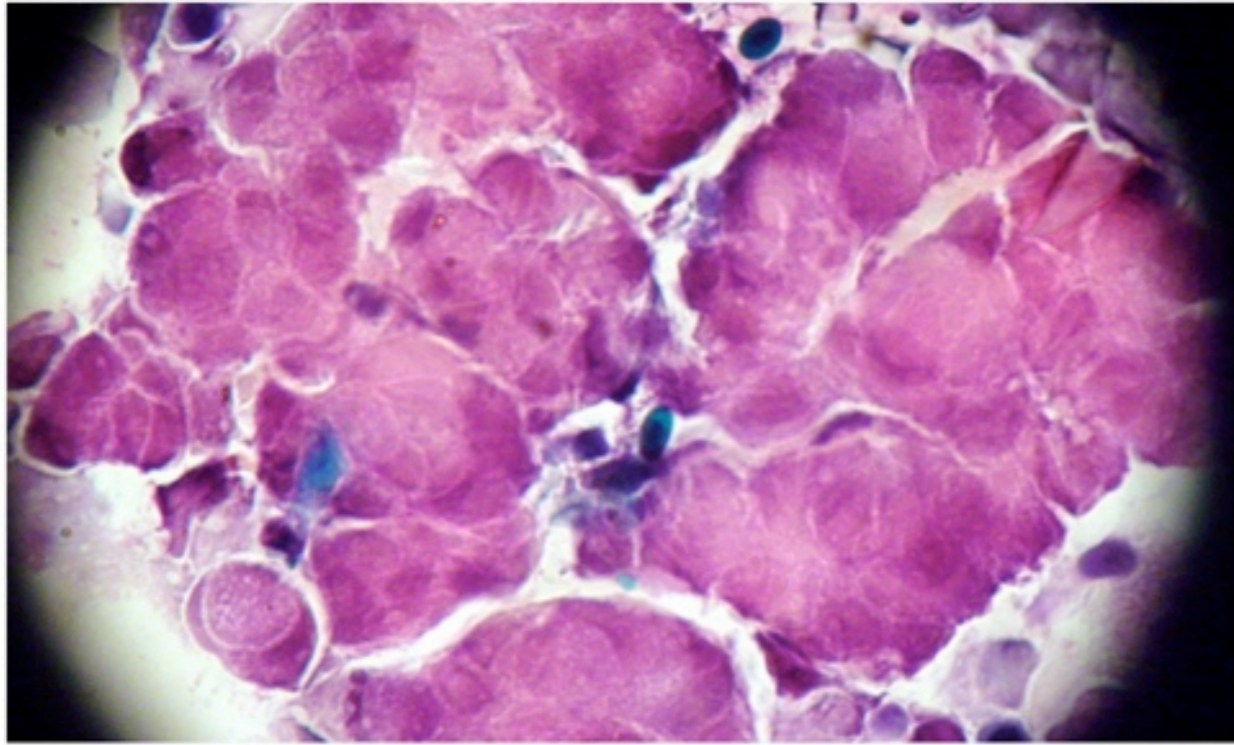
**Comparison between ATP and
MTT assays; 20 drugs tested;
5 adenocarcinomas**

In green is regression line +/- 95% CI

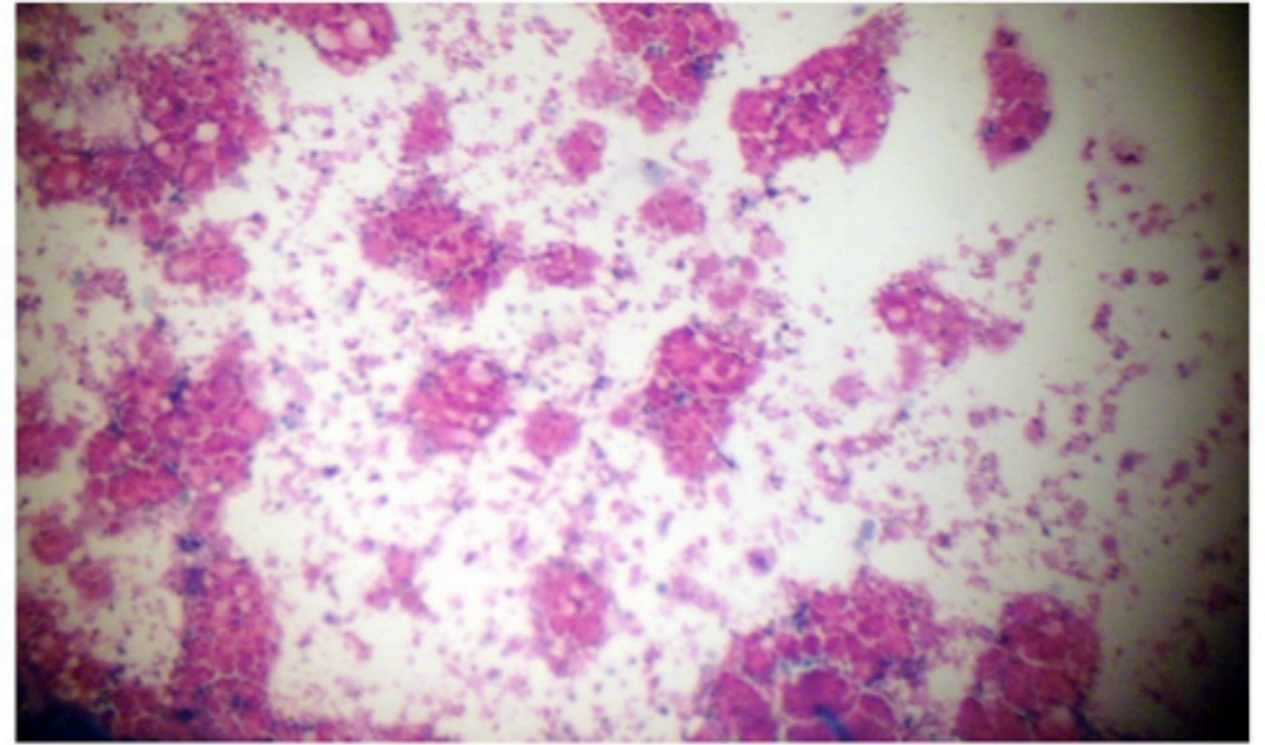


96 hr. DISC Assay vs 42 hr Caspase 3/7 expression
Ovarian Cancer, 16 drugs; 2 concentrations

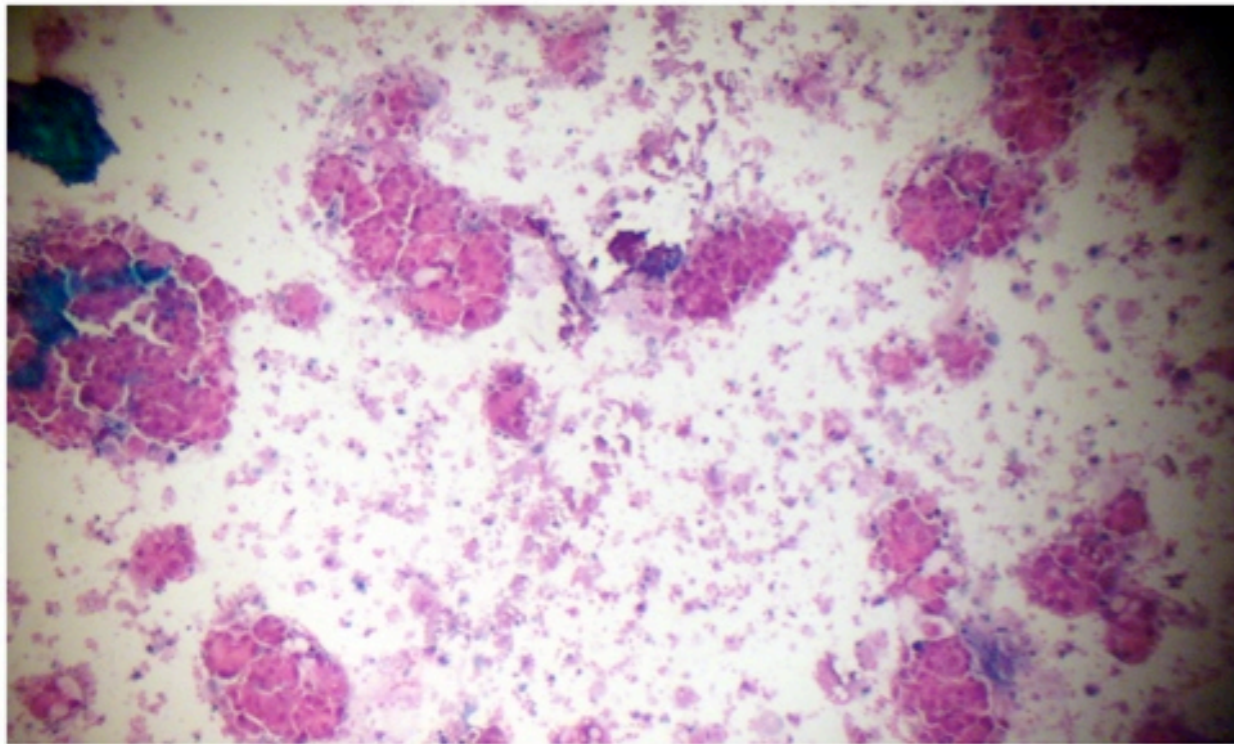




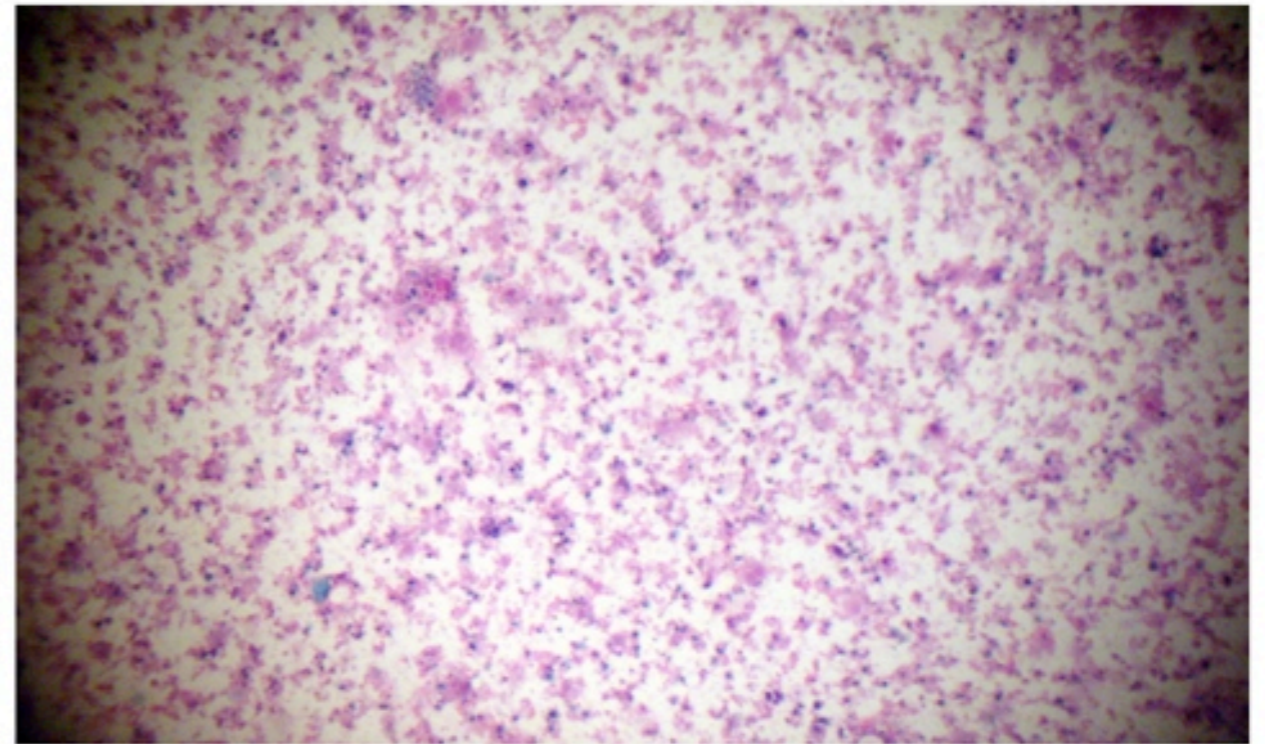
**Ovarian Preculture
400X**



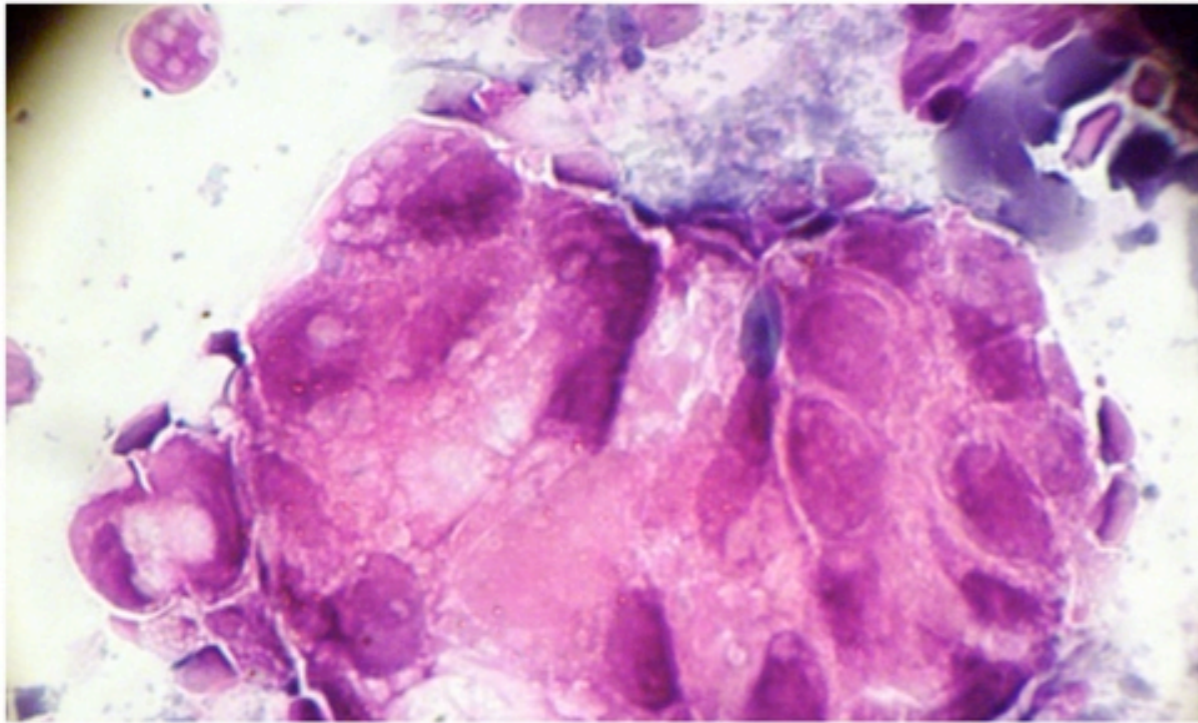
Postculture 40X



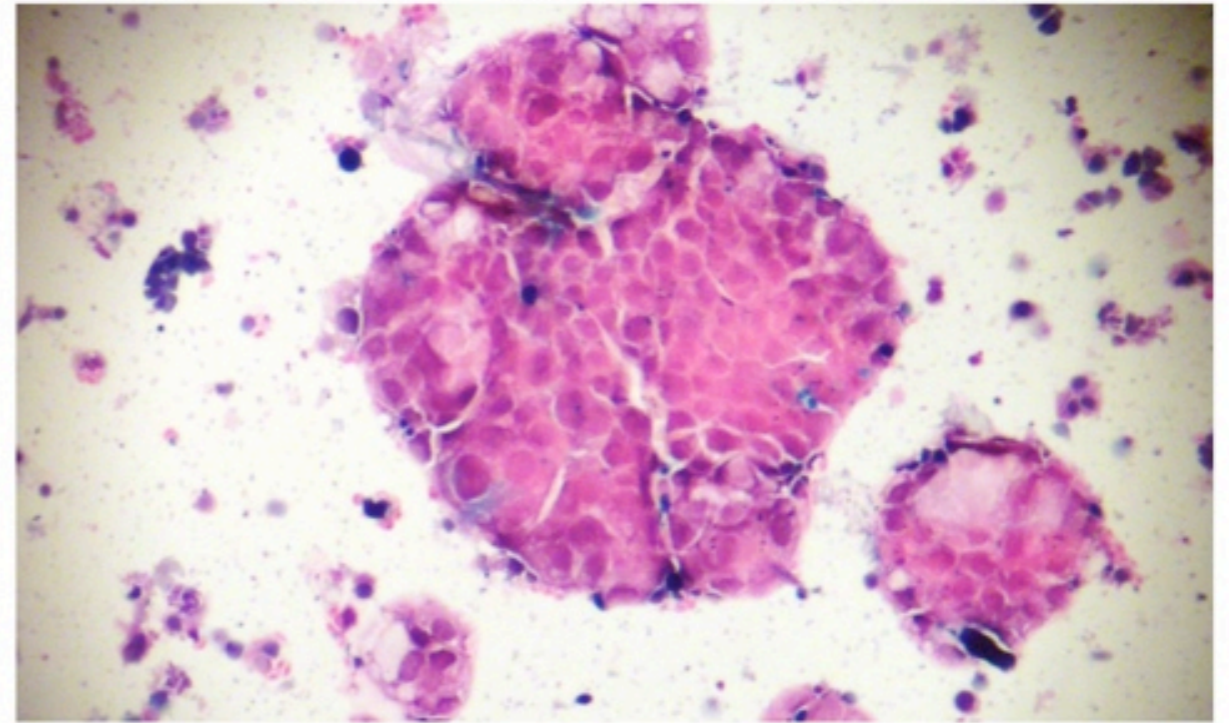
Doxil



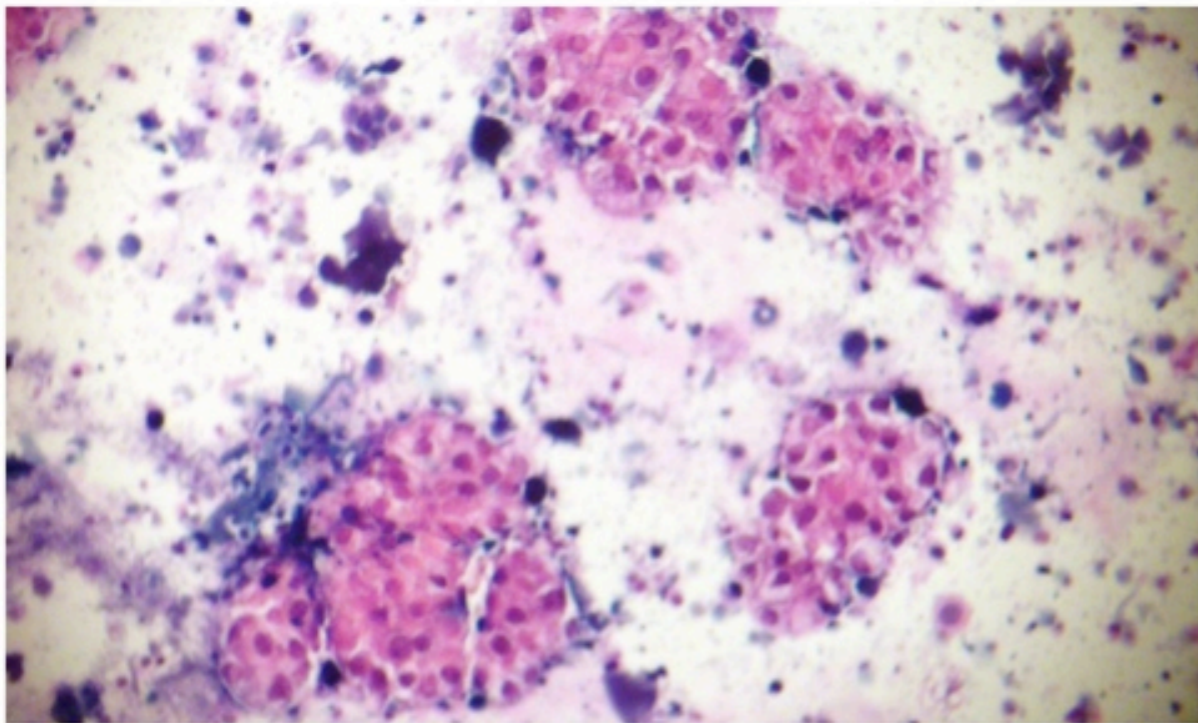
Topotecan



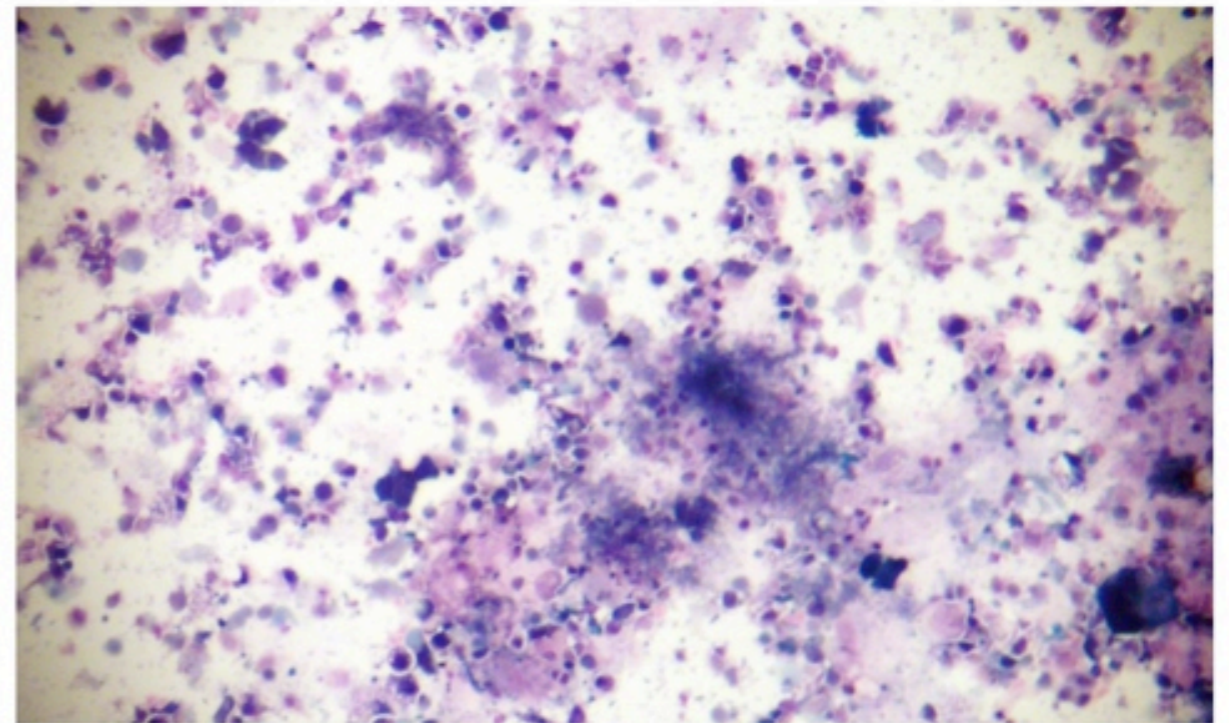
**NSCLC Preculture
400X**



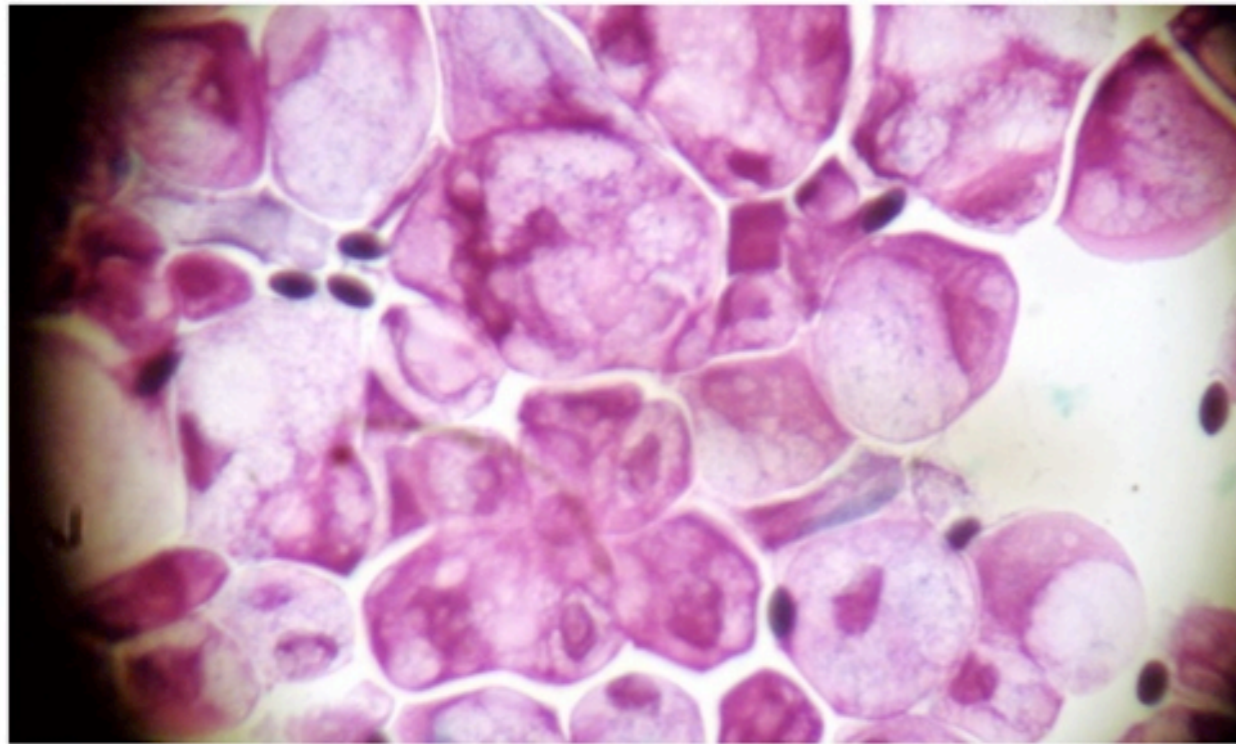
Postculture 100X



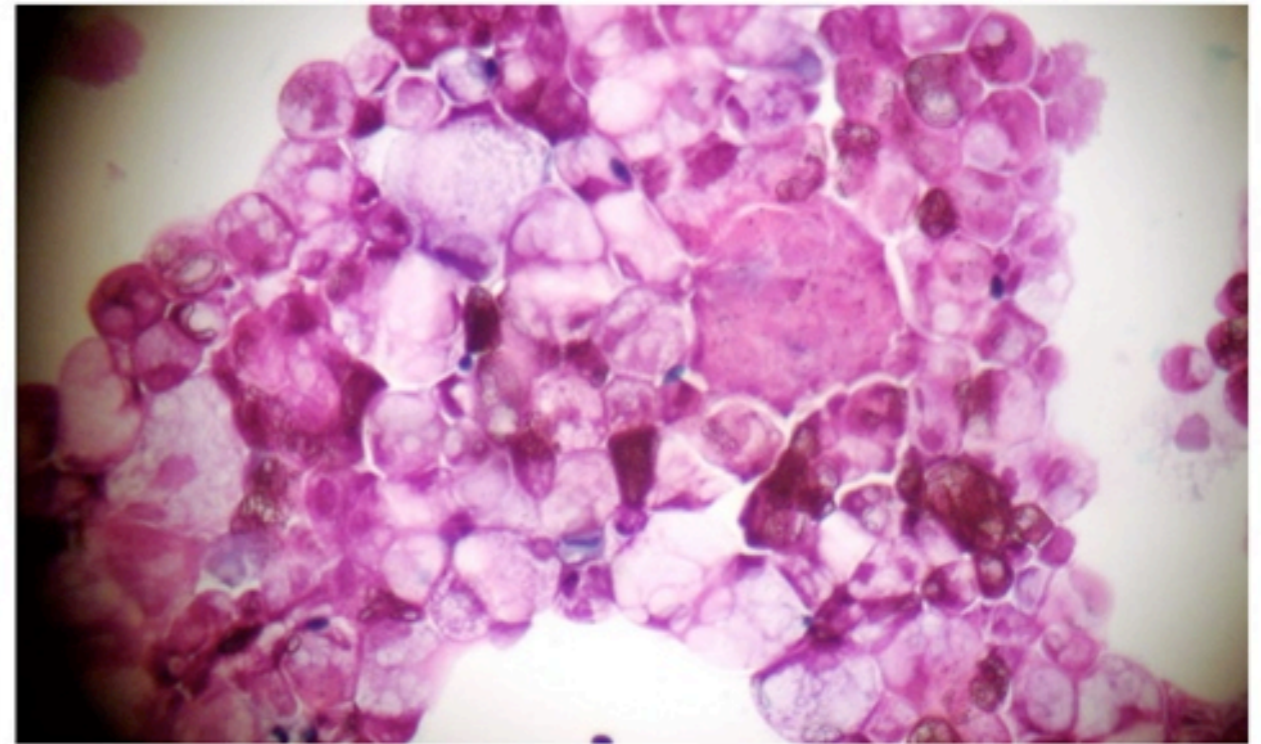
Docetaxel 100X



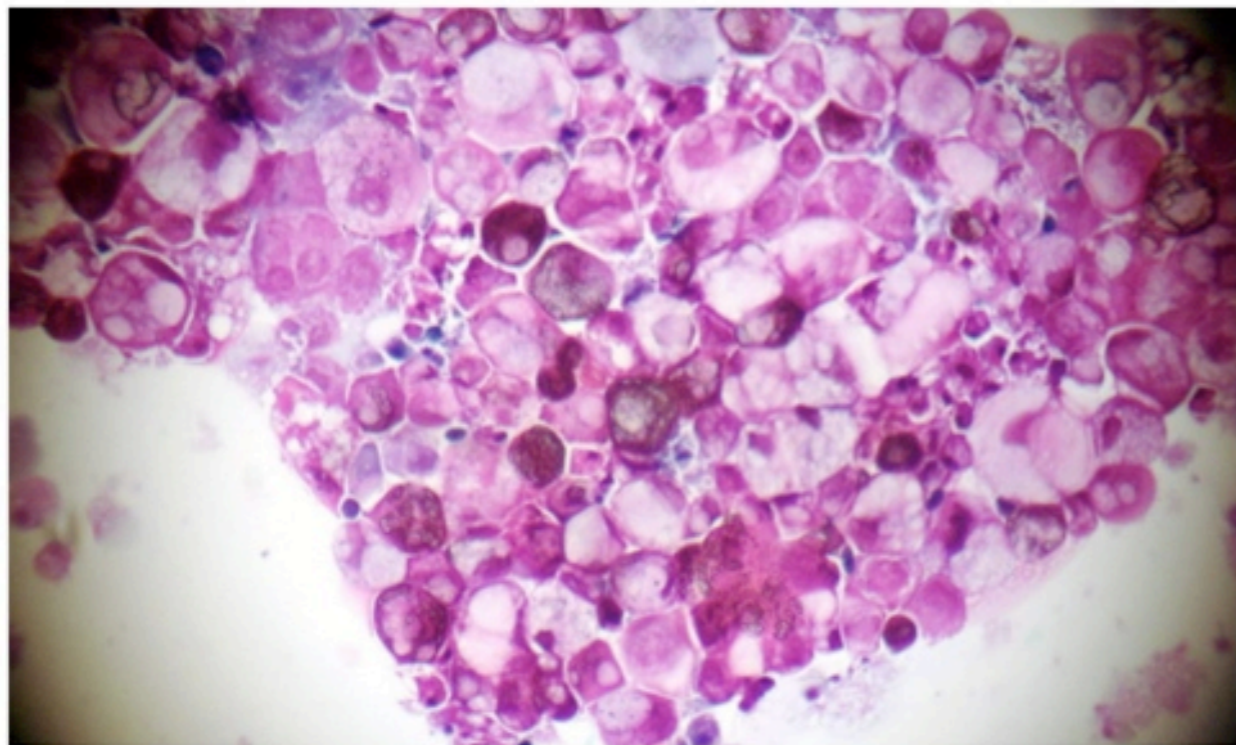
Cisplatin 100X



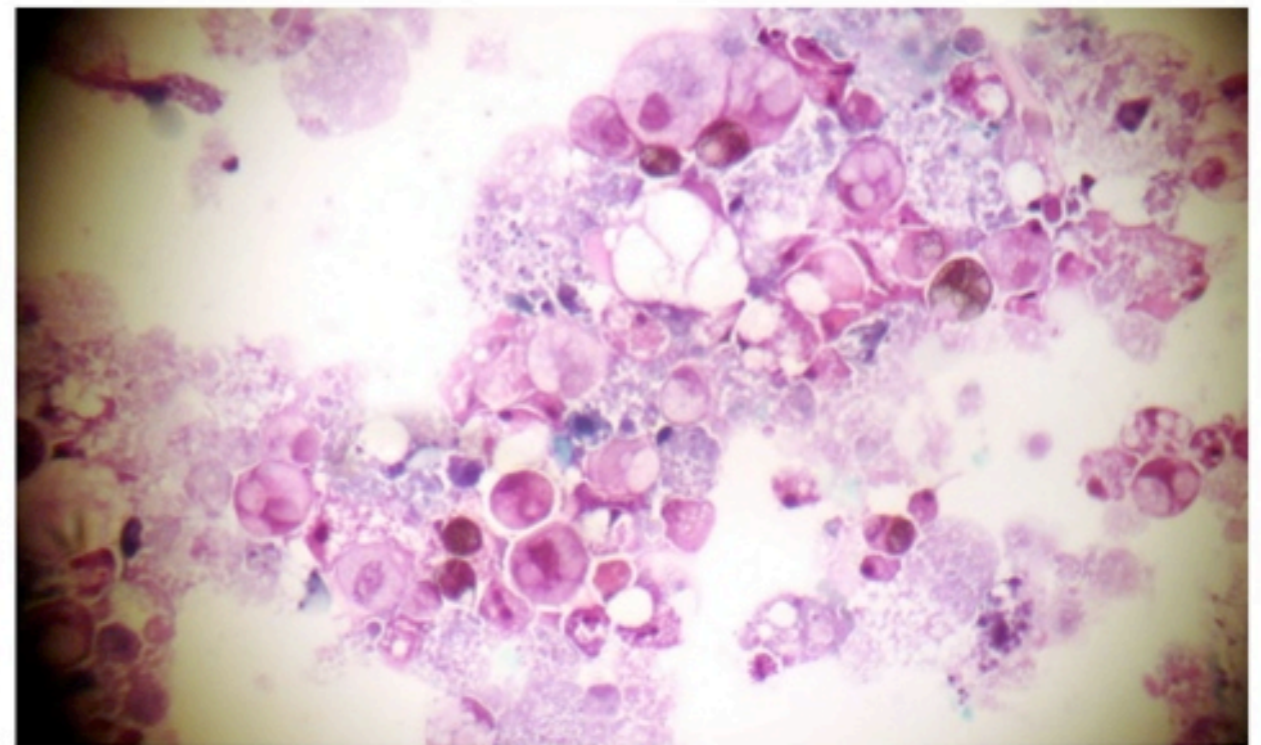
**Pancreatic Preculture
400X**



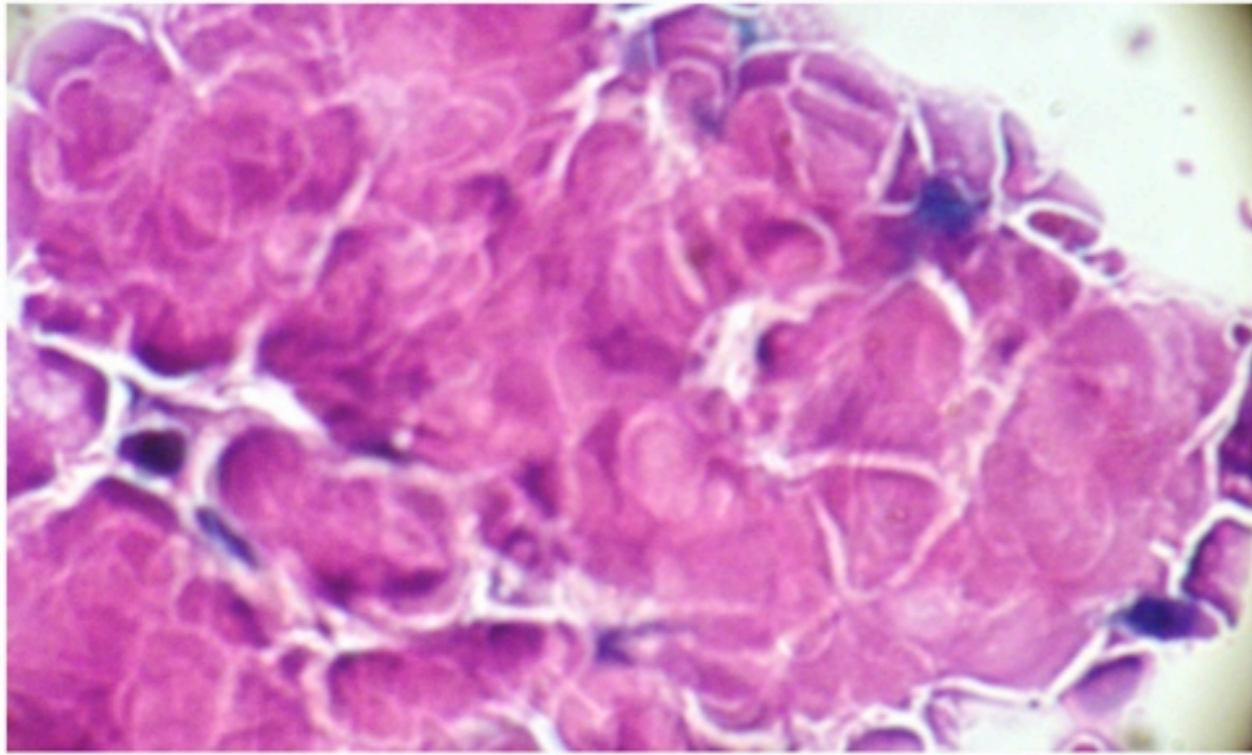
Postculture 200X



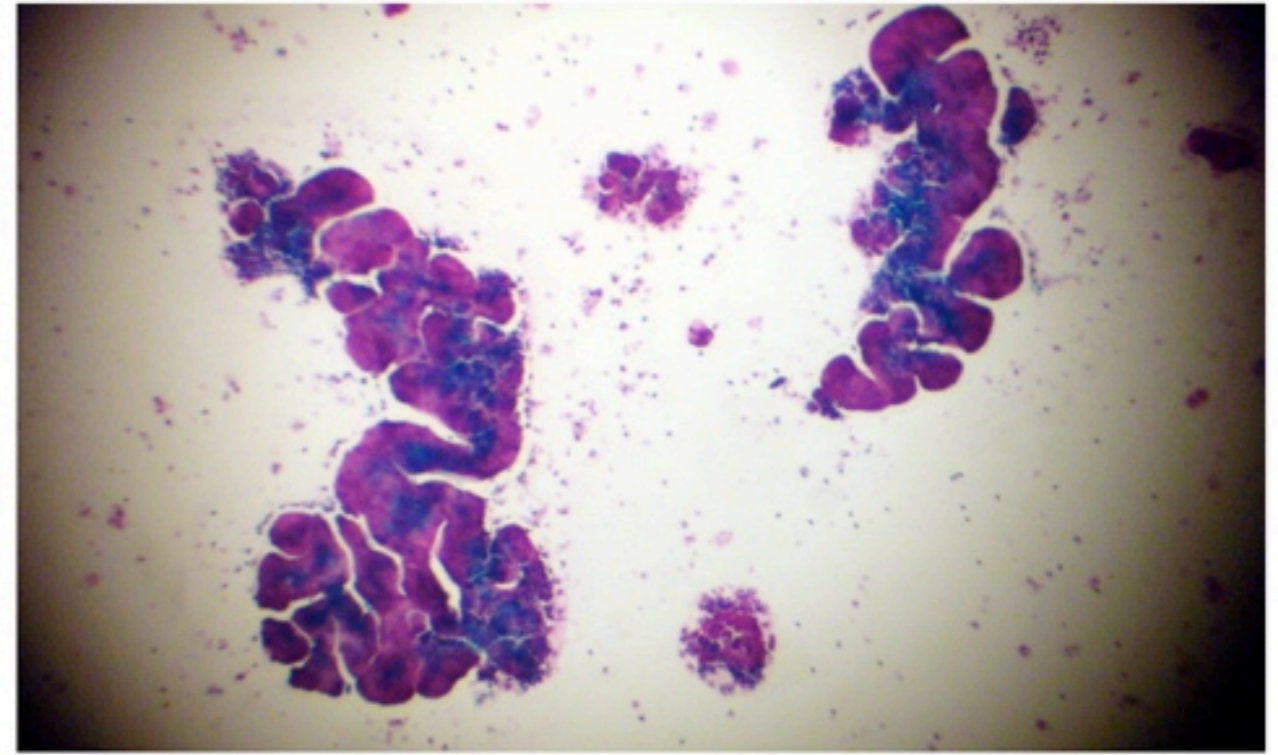
Cisplatin



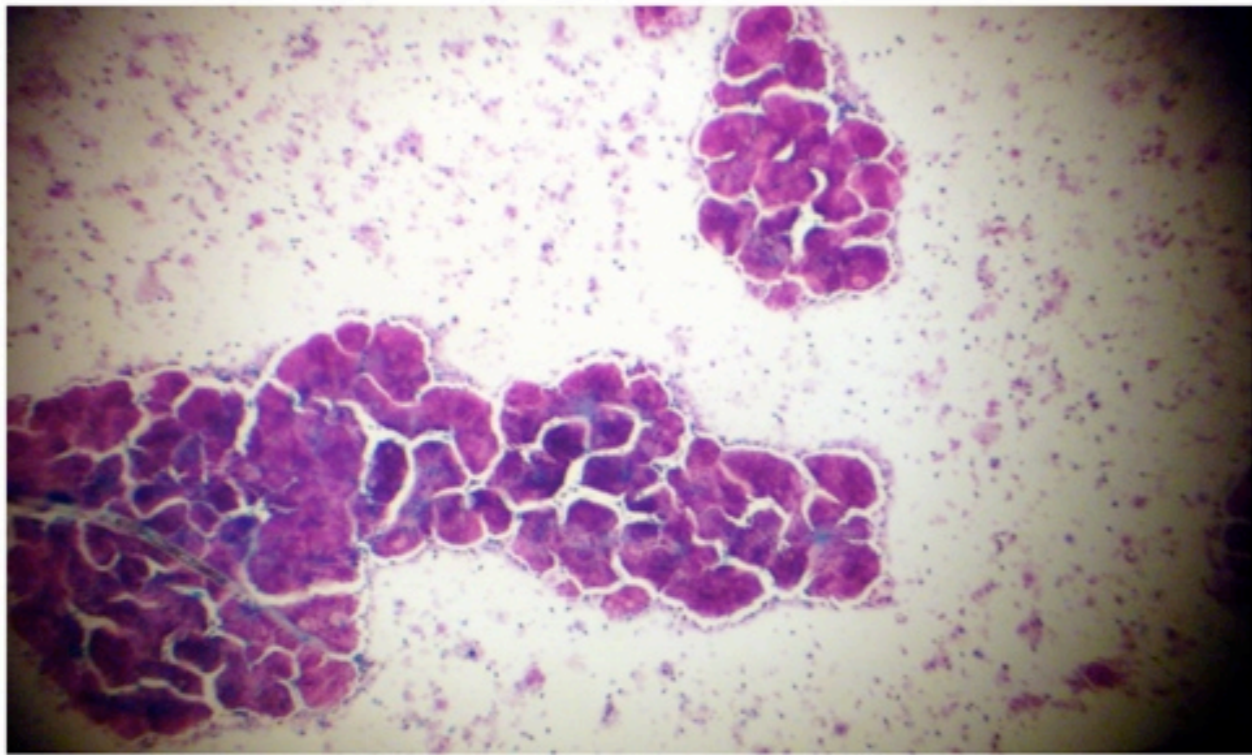
Gemcitabine+Cisplatin



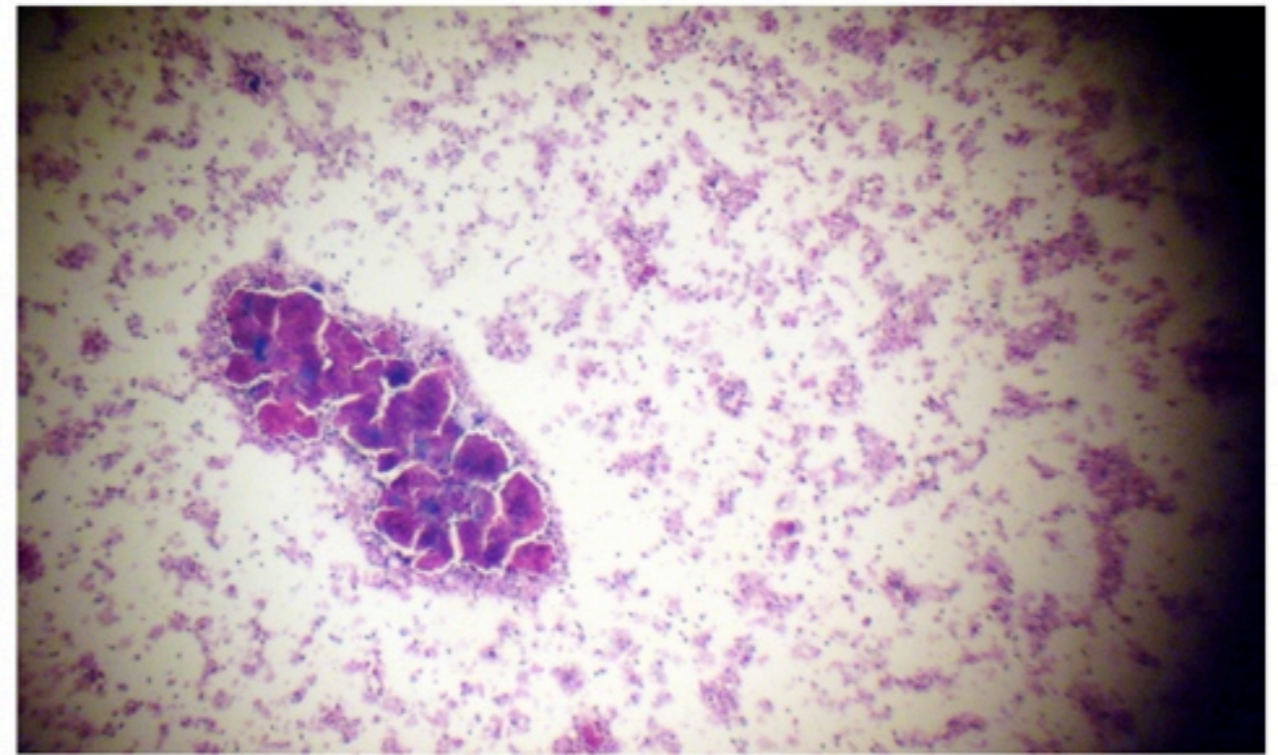
**Colon Cancer Preculture
400X**



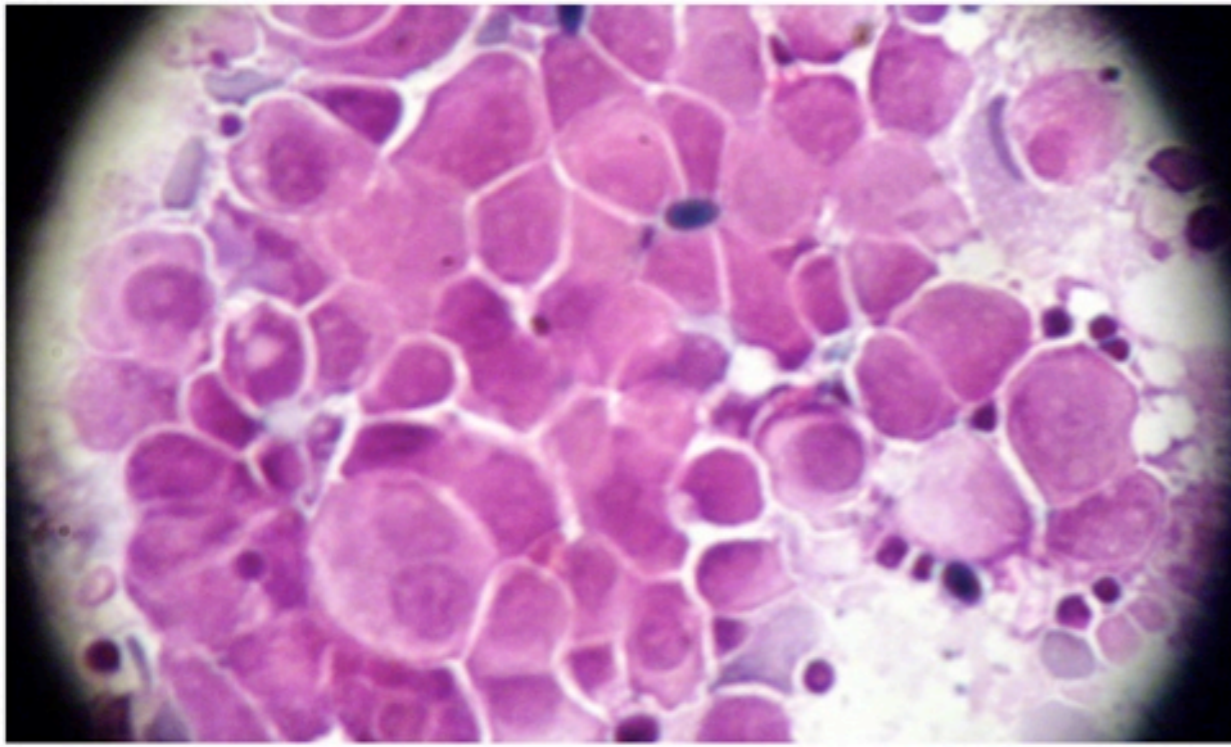
Postculture 40X



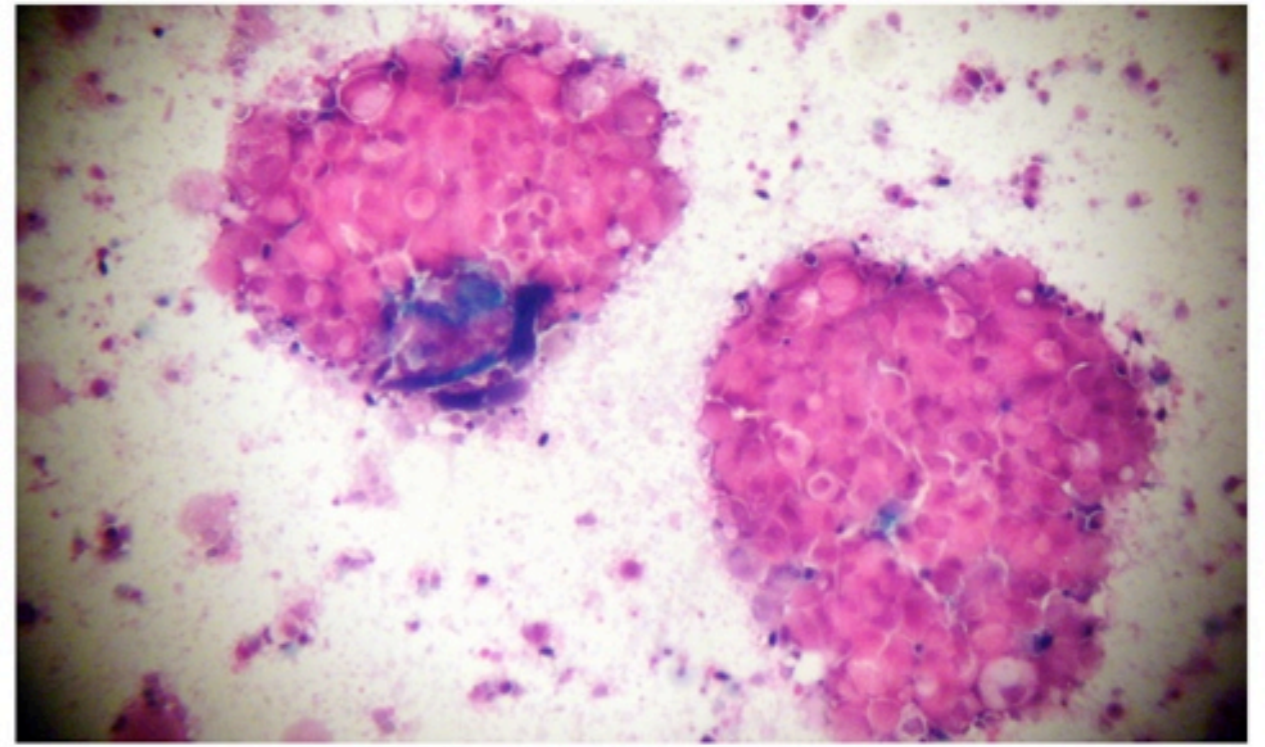
Oxaliplatin



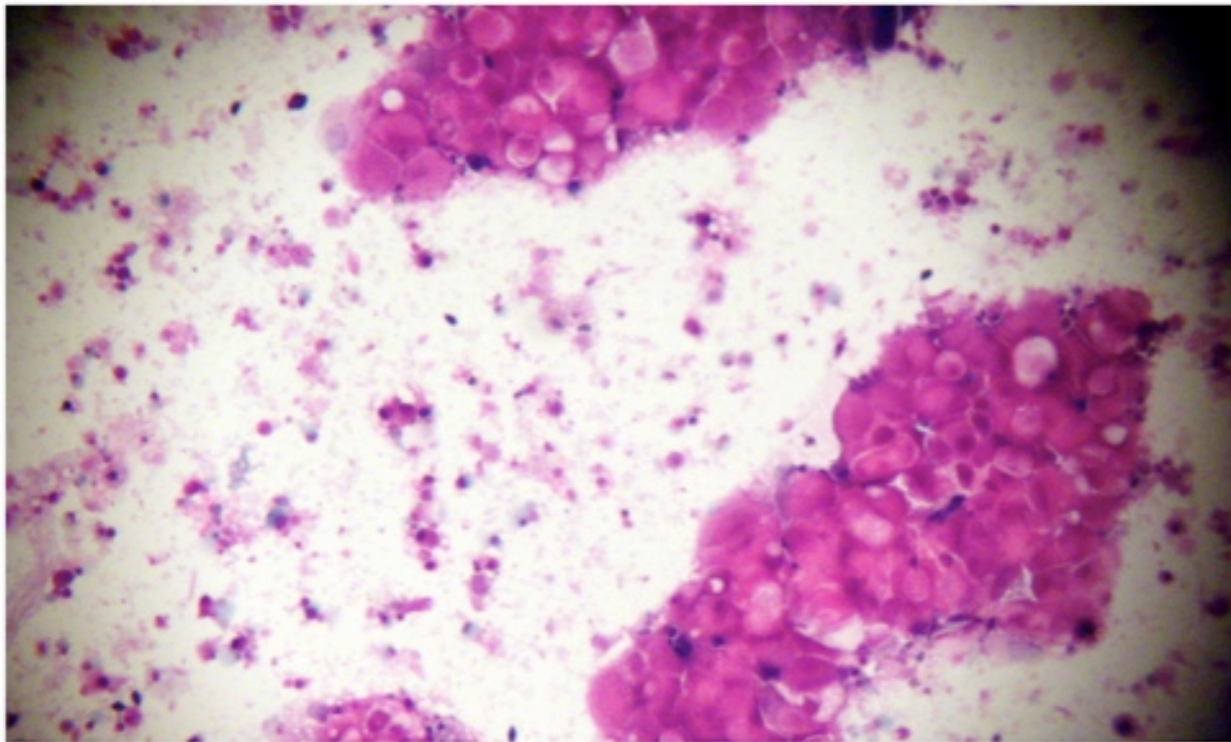
Gemcitabine+Oxaliplatin



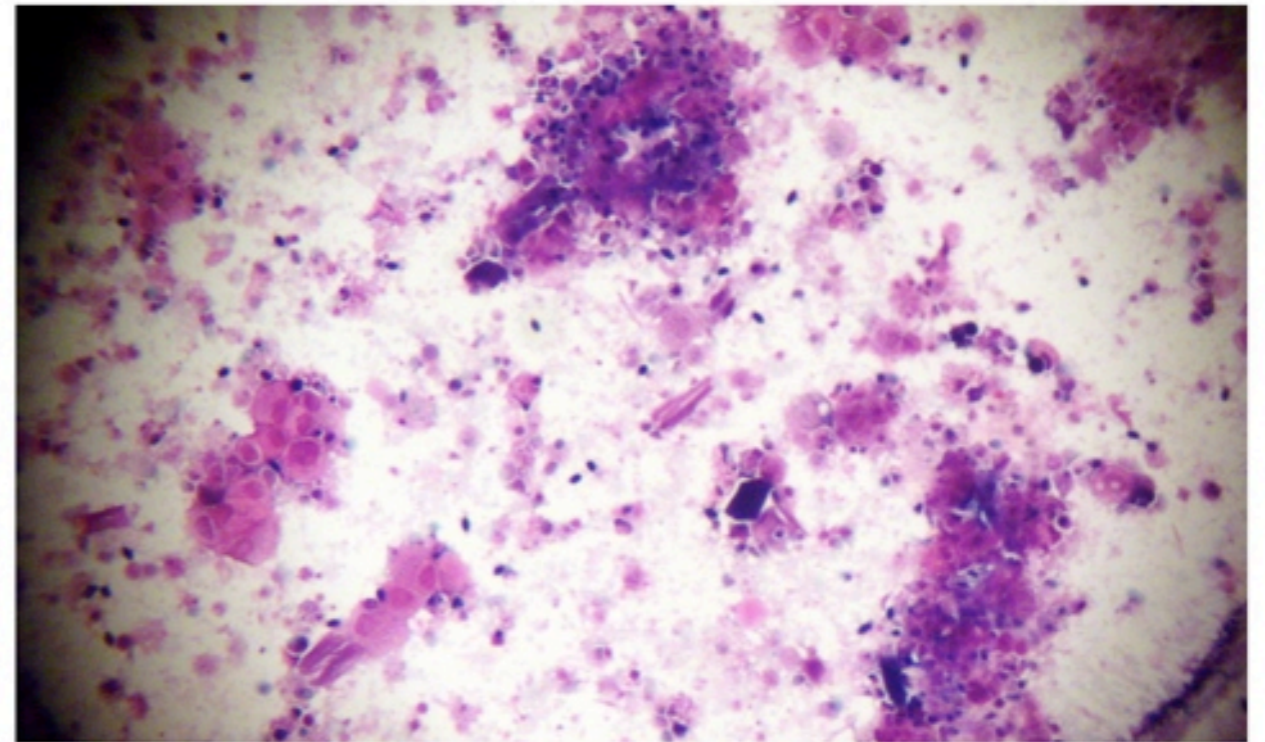
**Breast Cancer Preculture
400X**



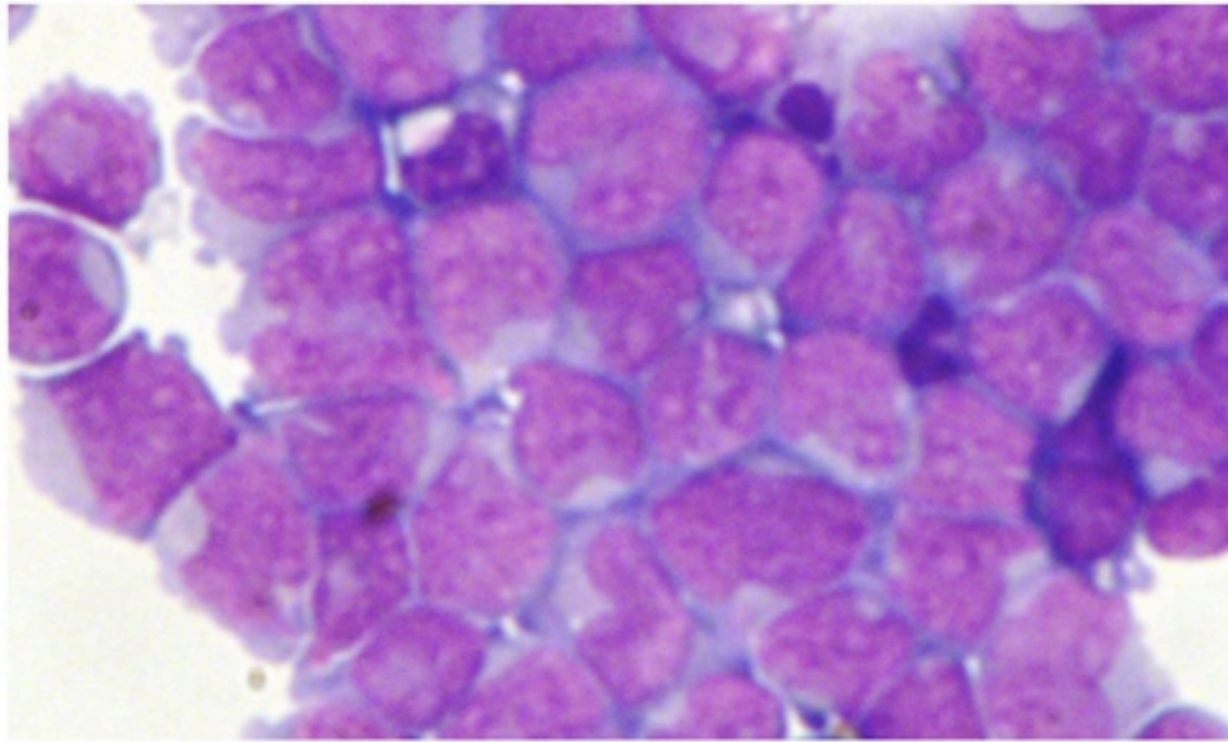
Postculture 100X



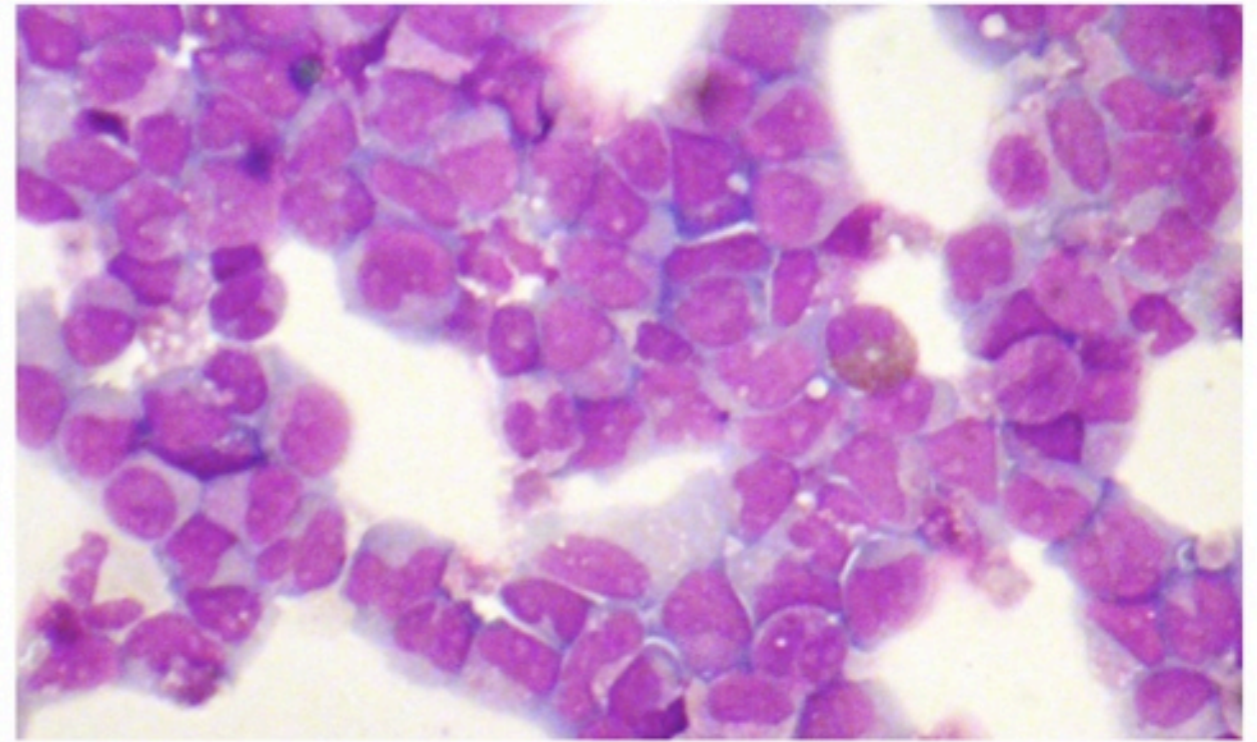
Cyclophosphamide (4HC)



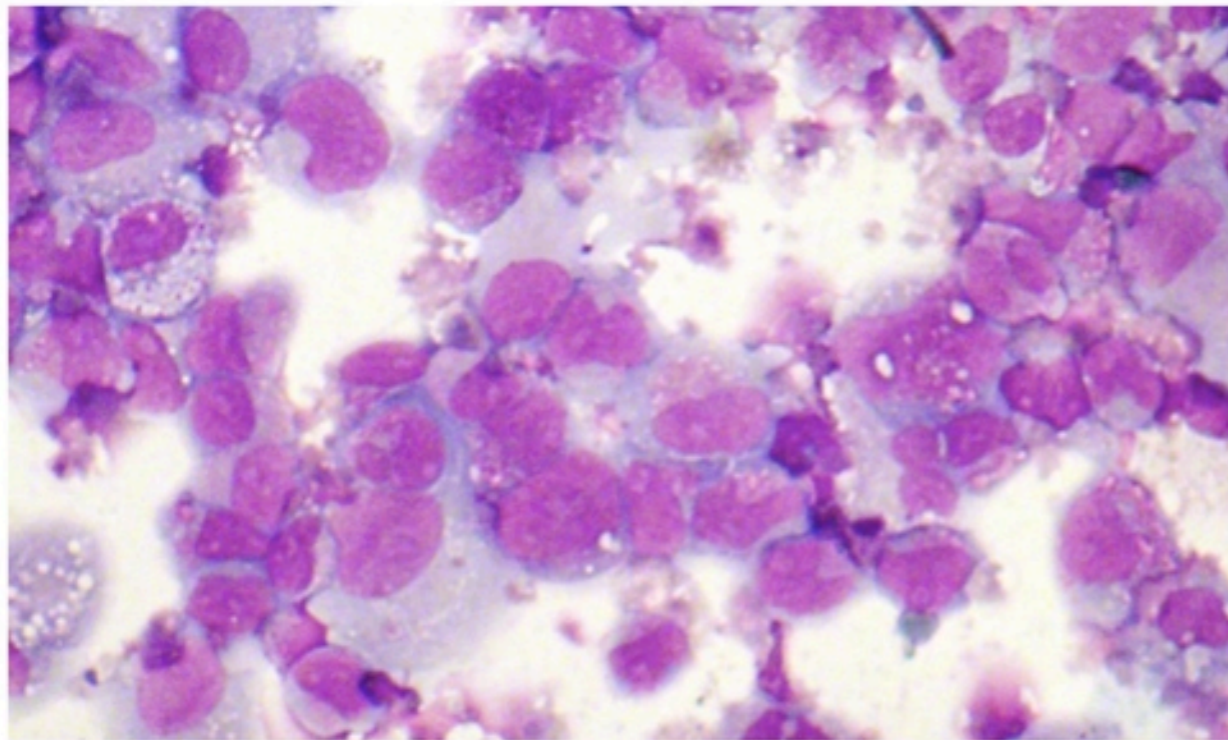
Vinorelbine+Tamoxifen



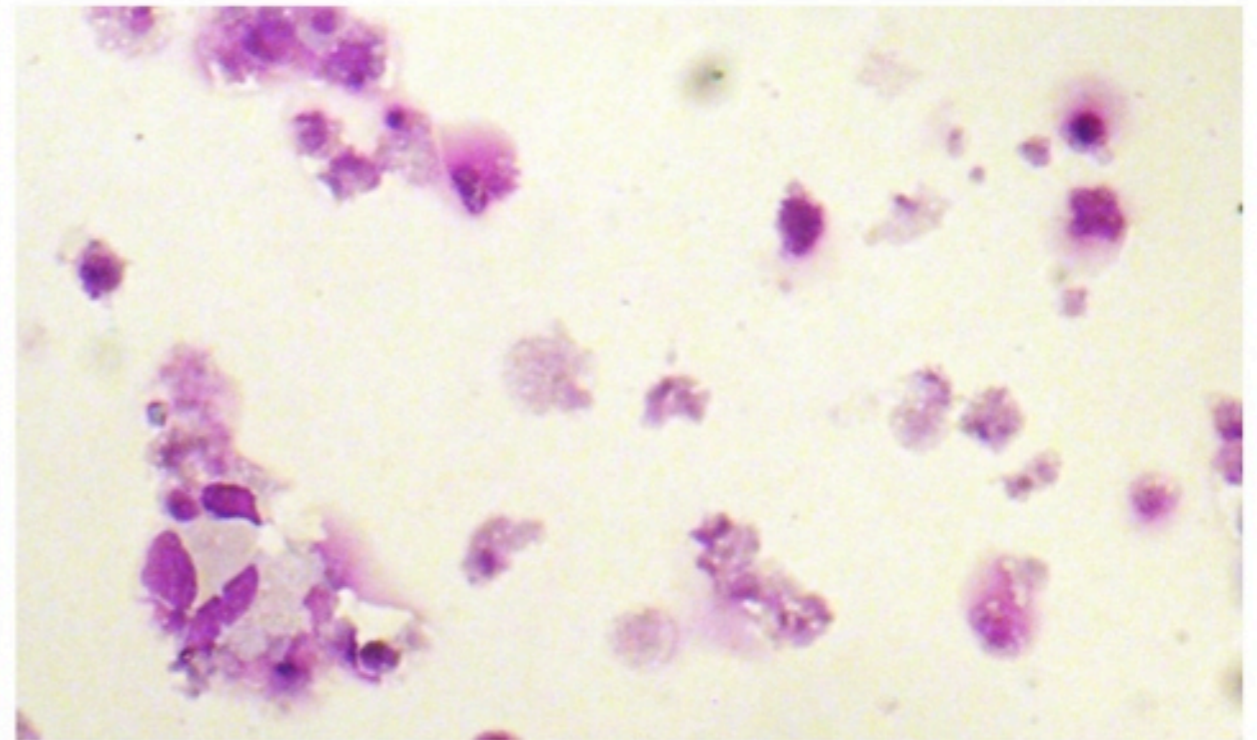
**Non-Hodgkin's Preculture
400X**



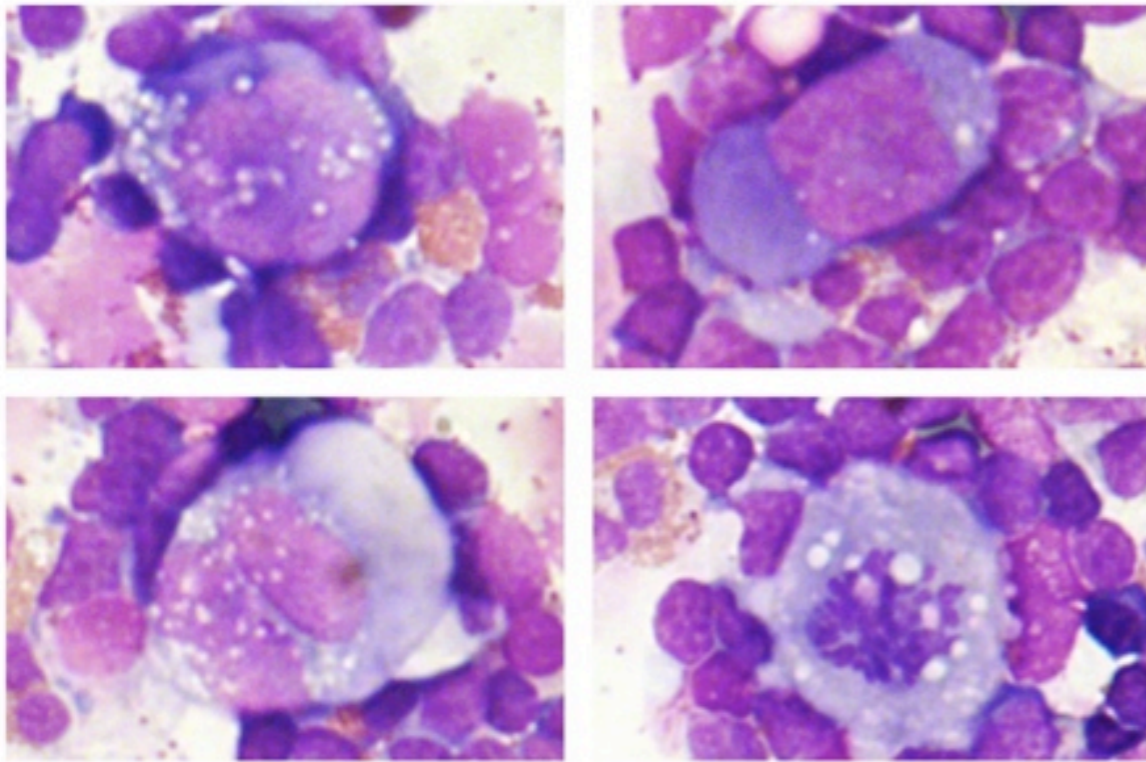
Postculture 200X



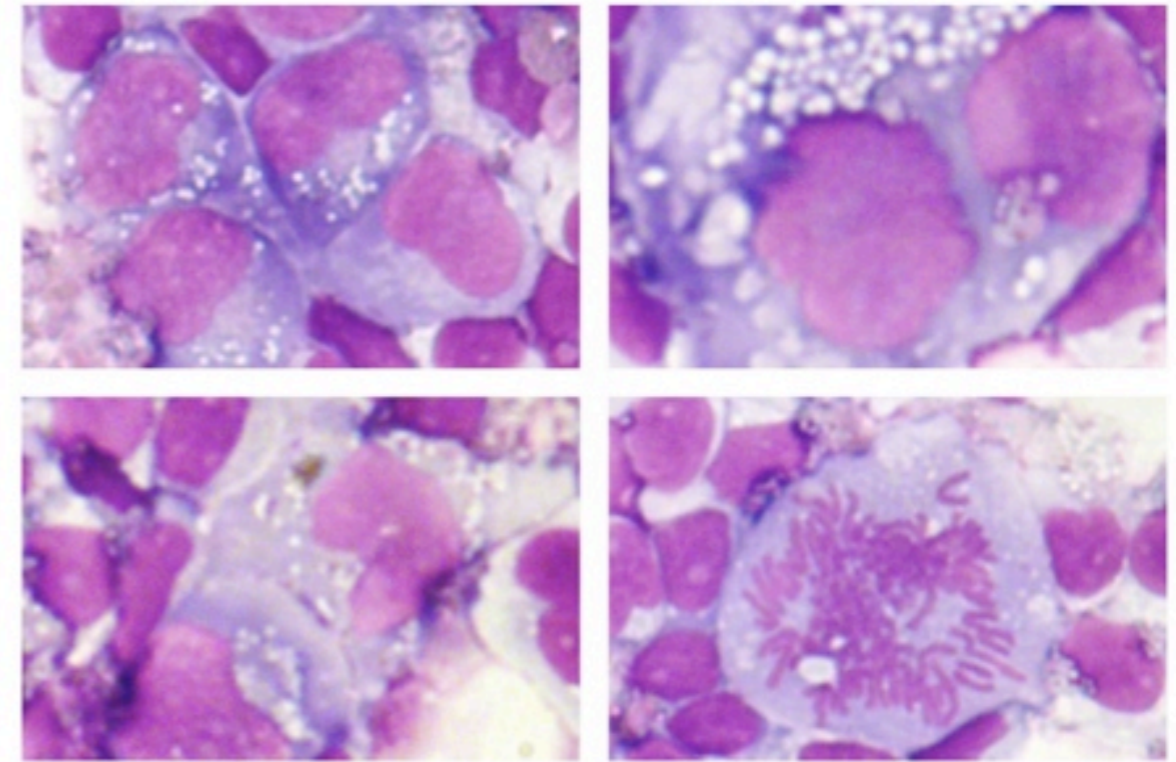
Doxorubicin



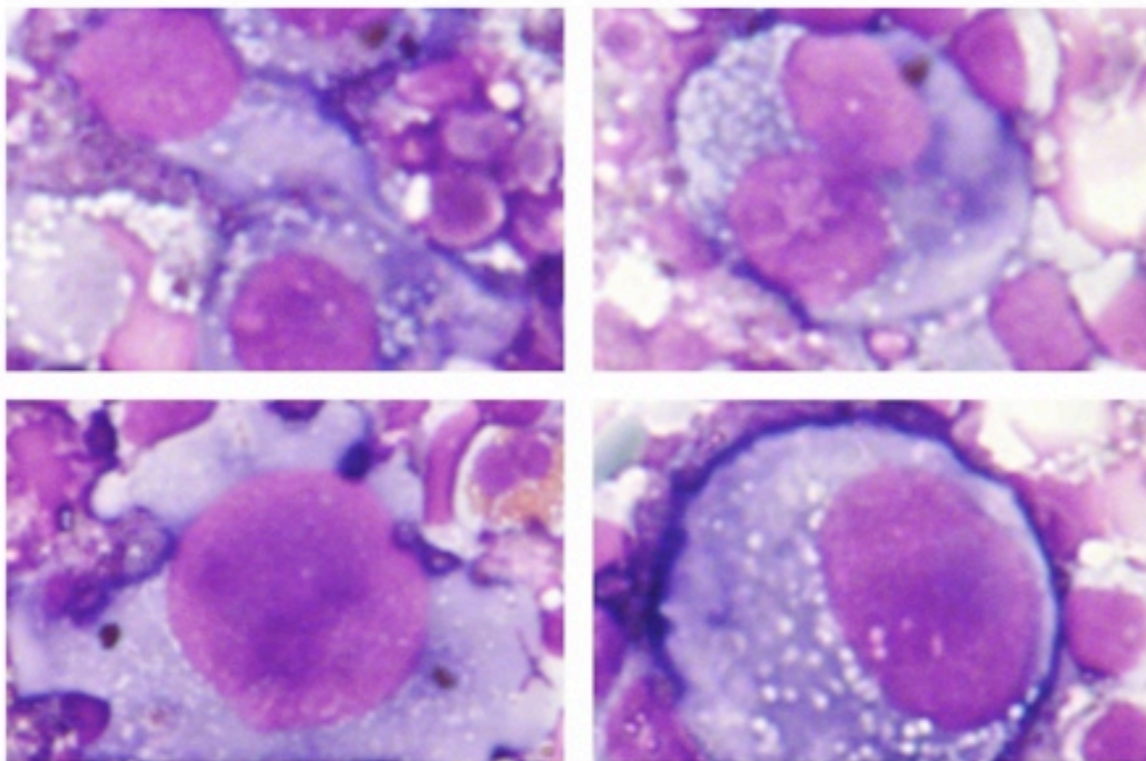
Fludarabine



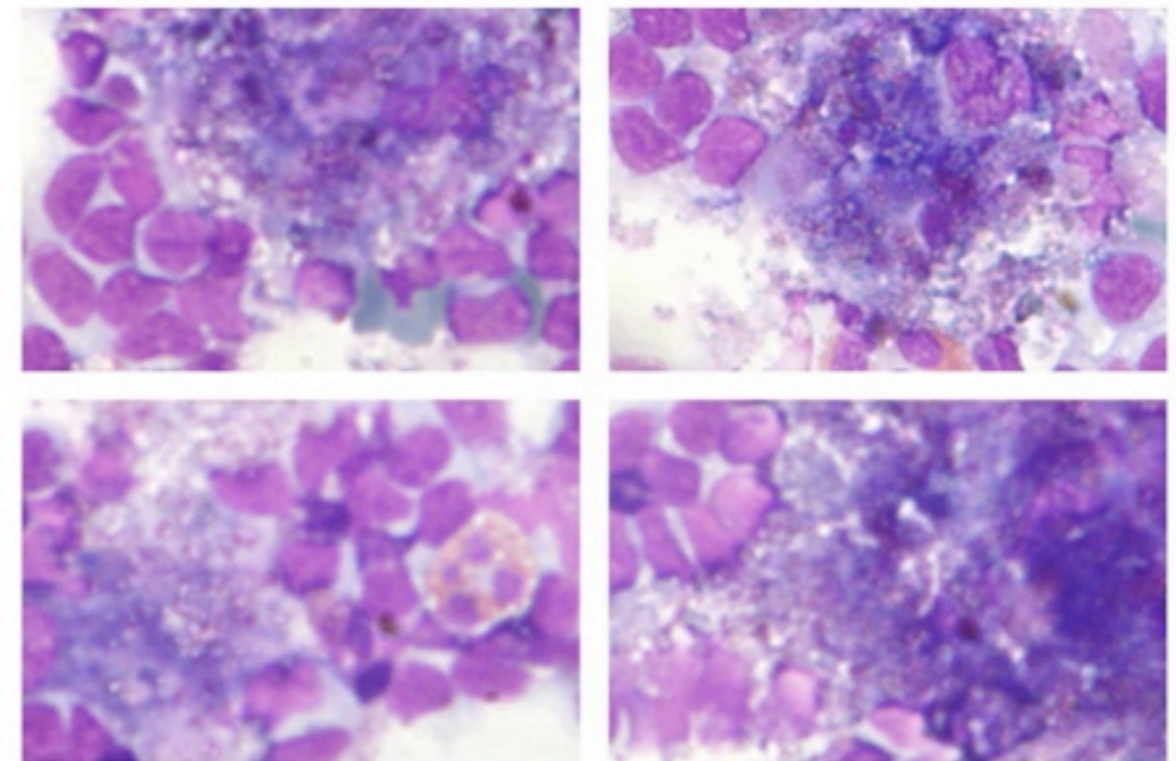
Hodgkin's Preculture



Hodgkin's Postculture



Cyclophosphamide (4HC)

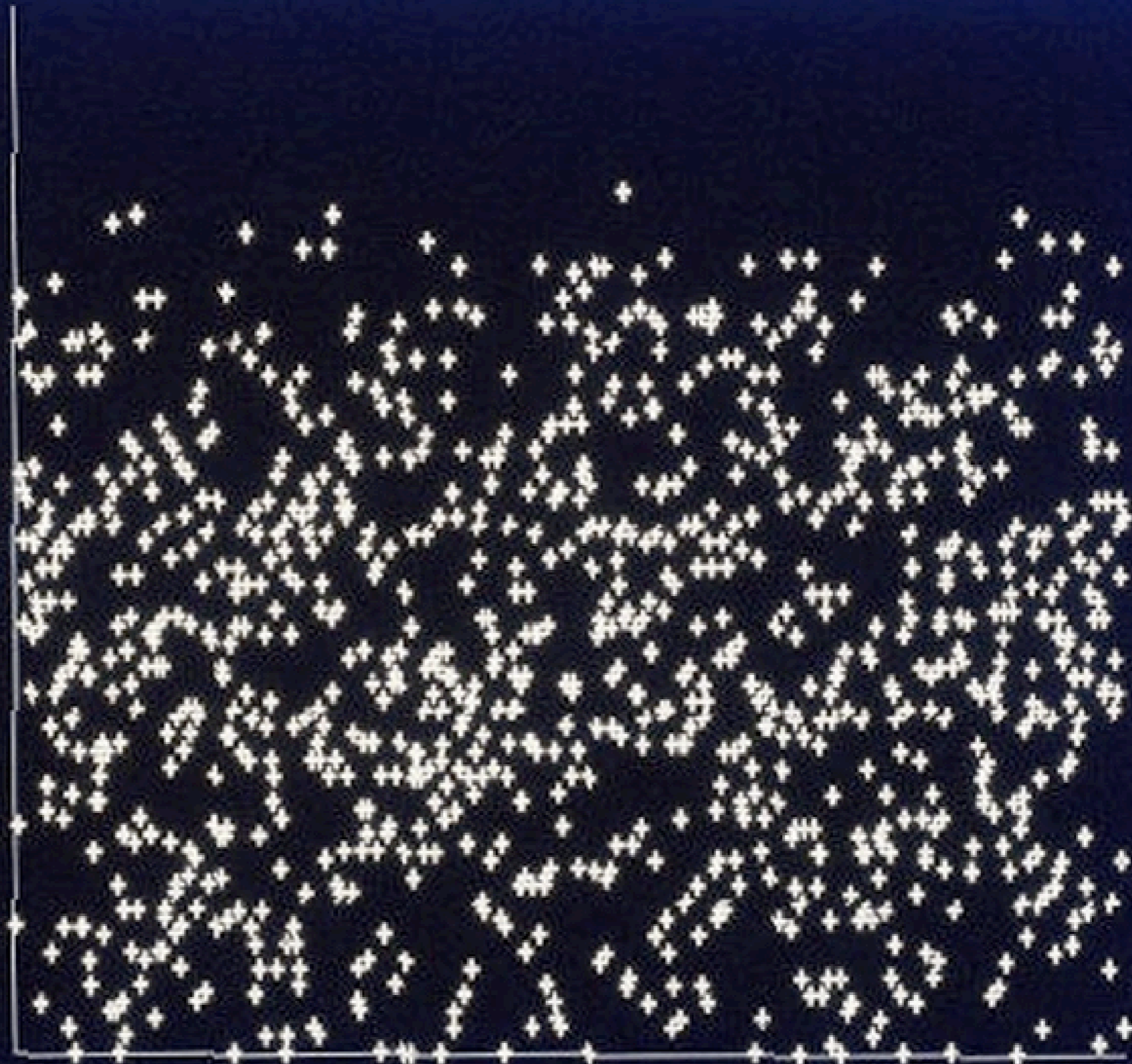


Irinotecan

Cisplatin Activity in 1,000 Randomly-Selected Fresh Tumor MTT Assays

Percent Control Cell Survival

100
75
50
25
0



Cisplatin Activity in 1,000 Randomly-Selected Fresh Tumor MTT Assays

Percent Control Cell Survival

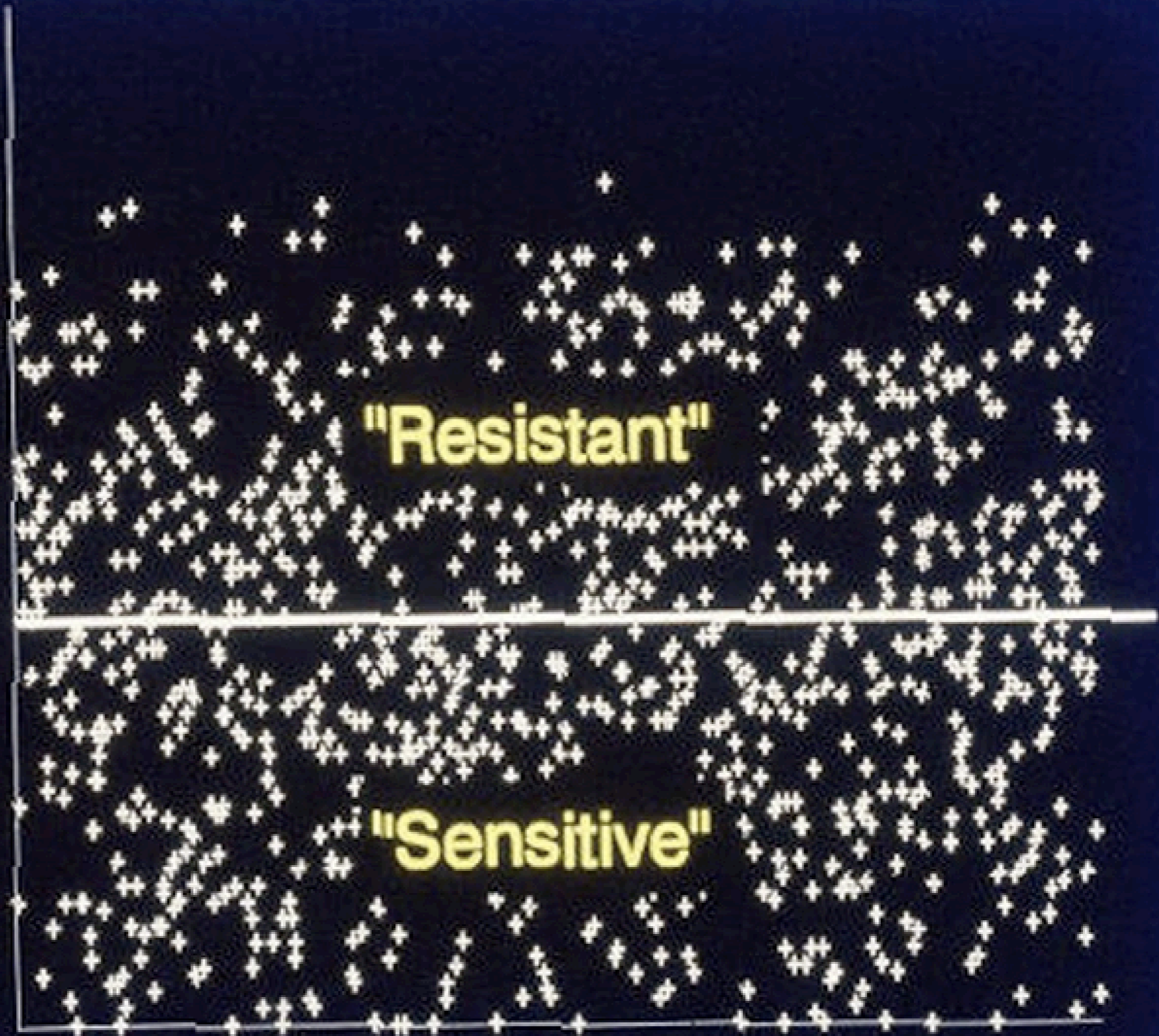
100

75

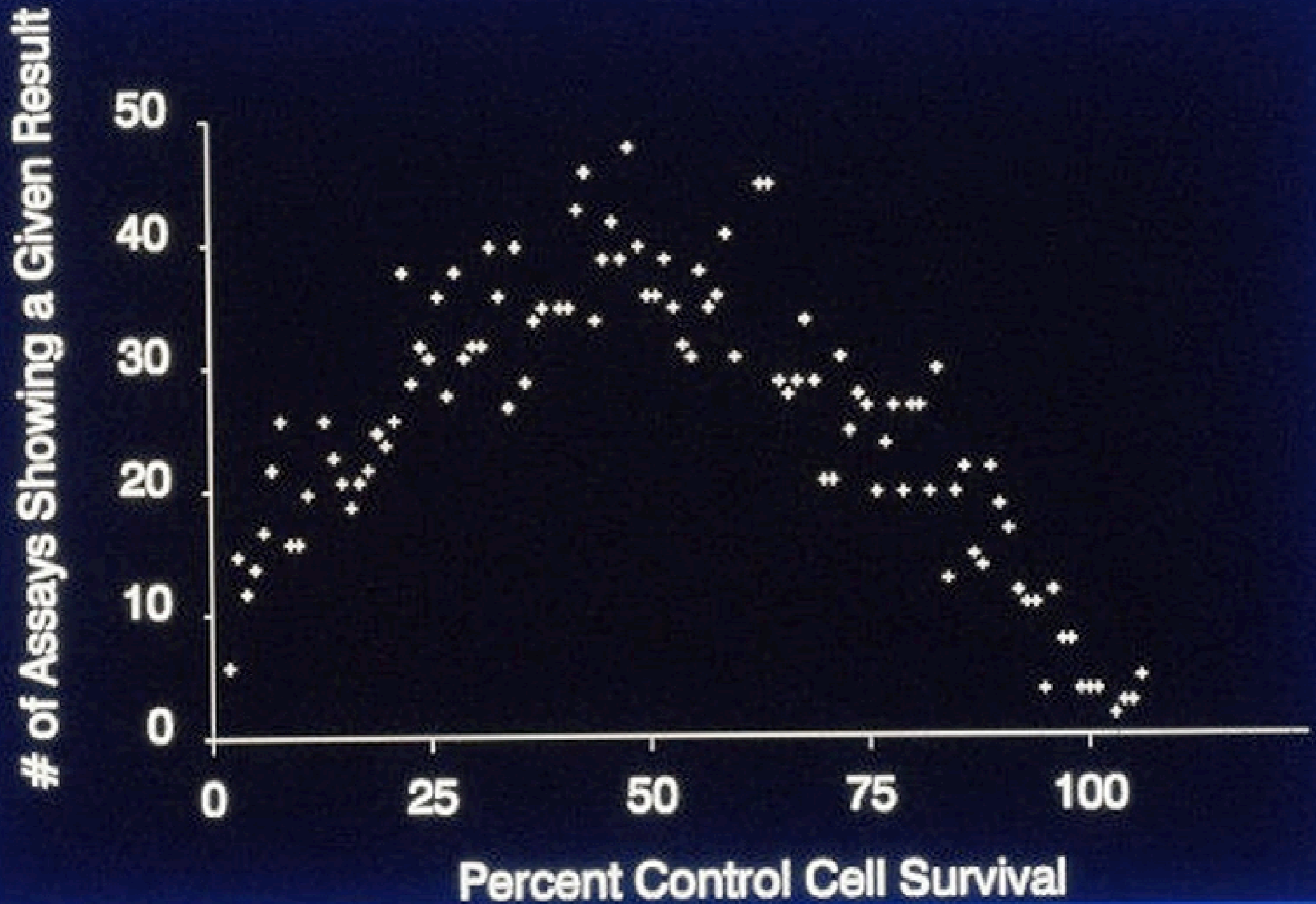
50

25

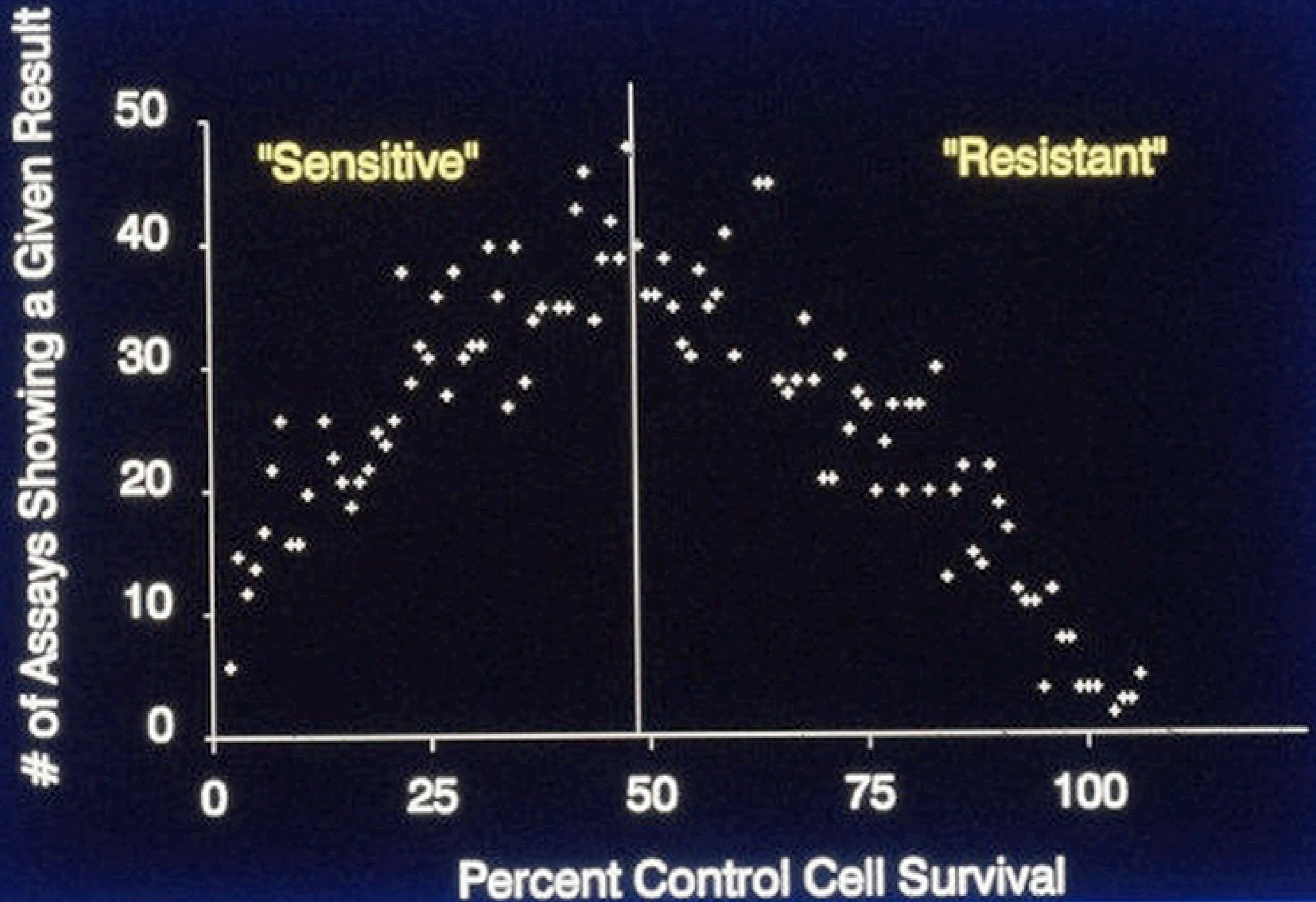
0



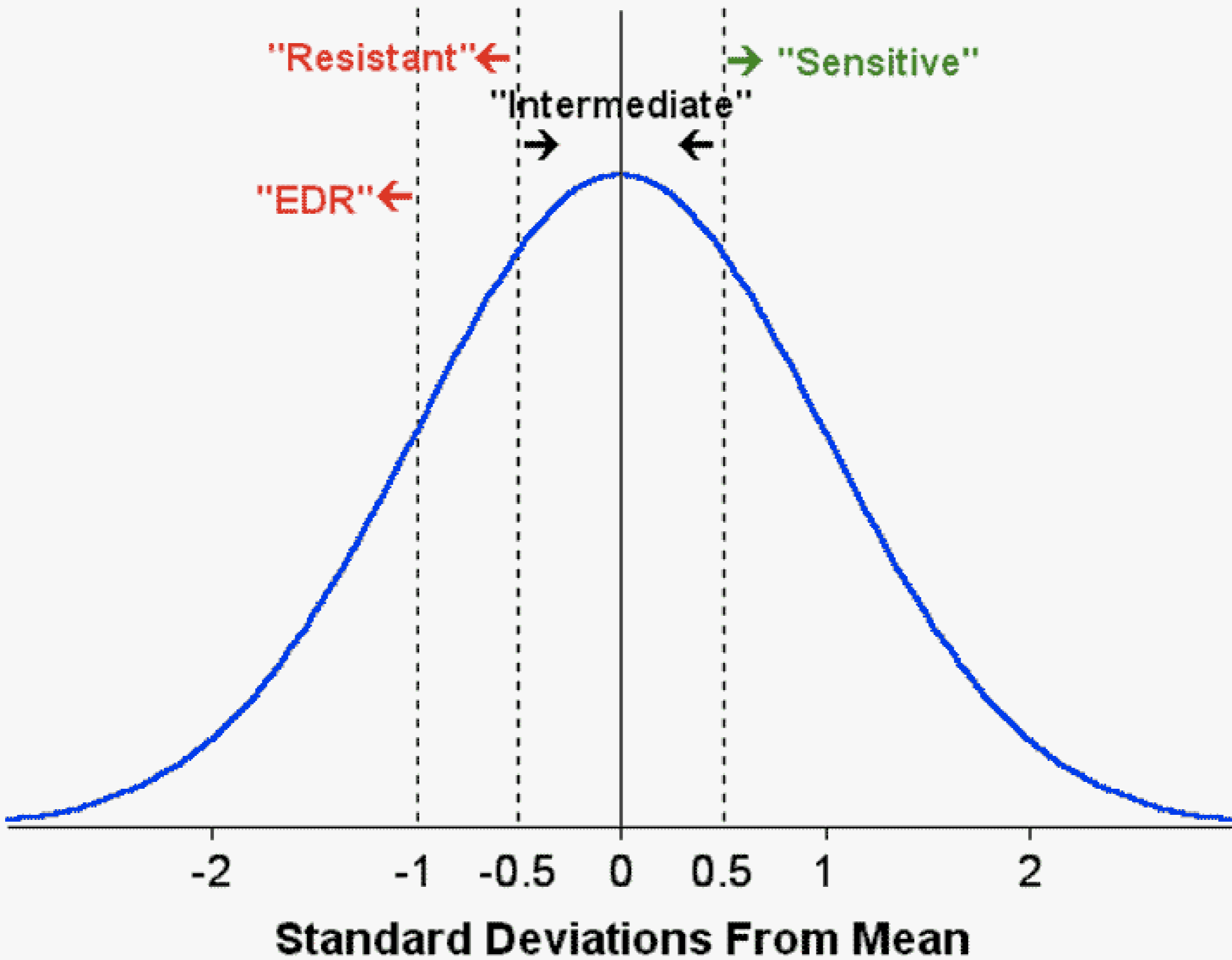
Distribution of Cisplatin Activity in 2,900 Fresh Tumor MTT Assays



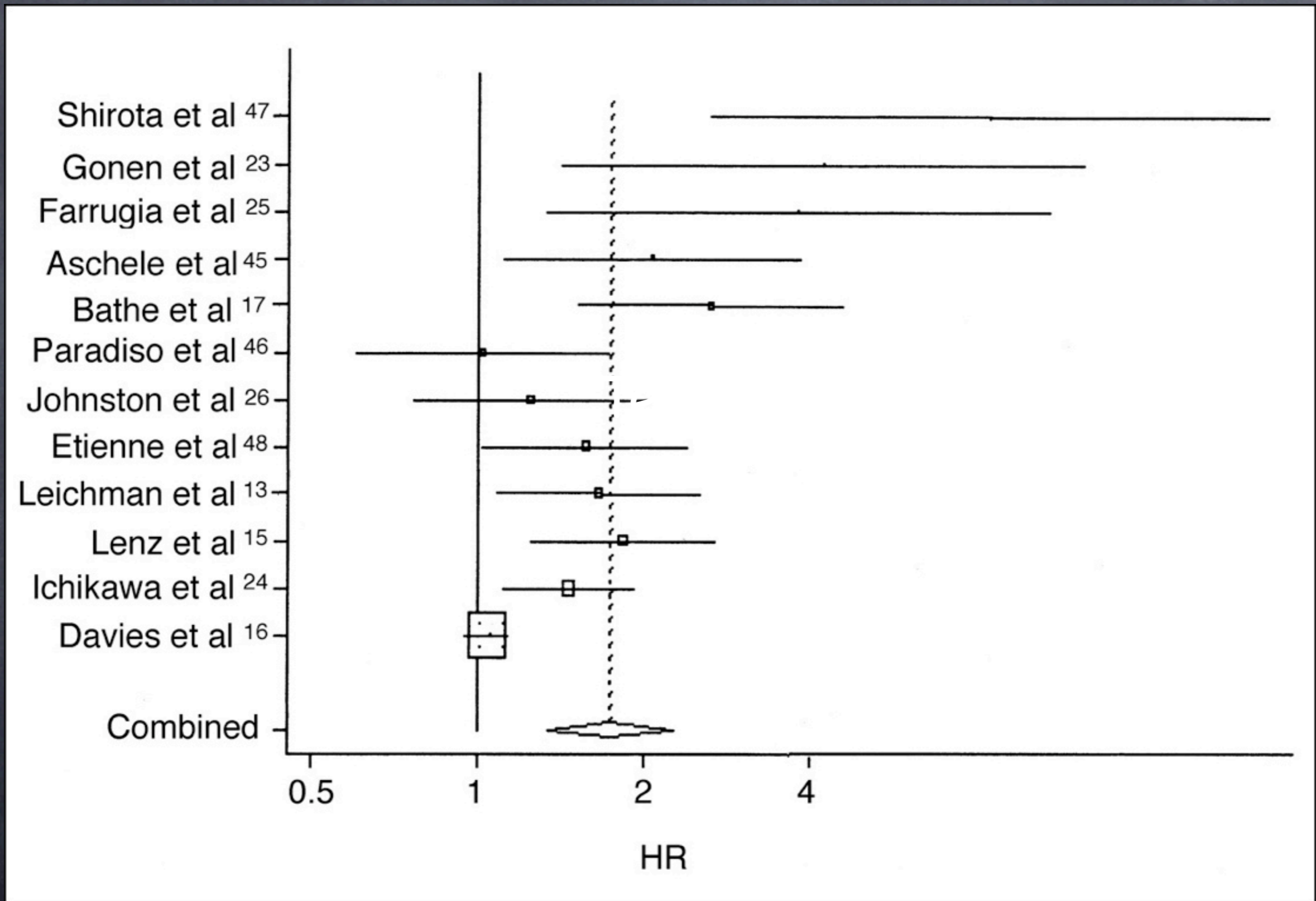
Distribution of Cisplatin Activity in 2,900 Fresh Tumor MTT Assays



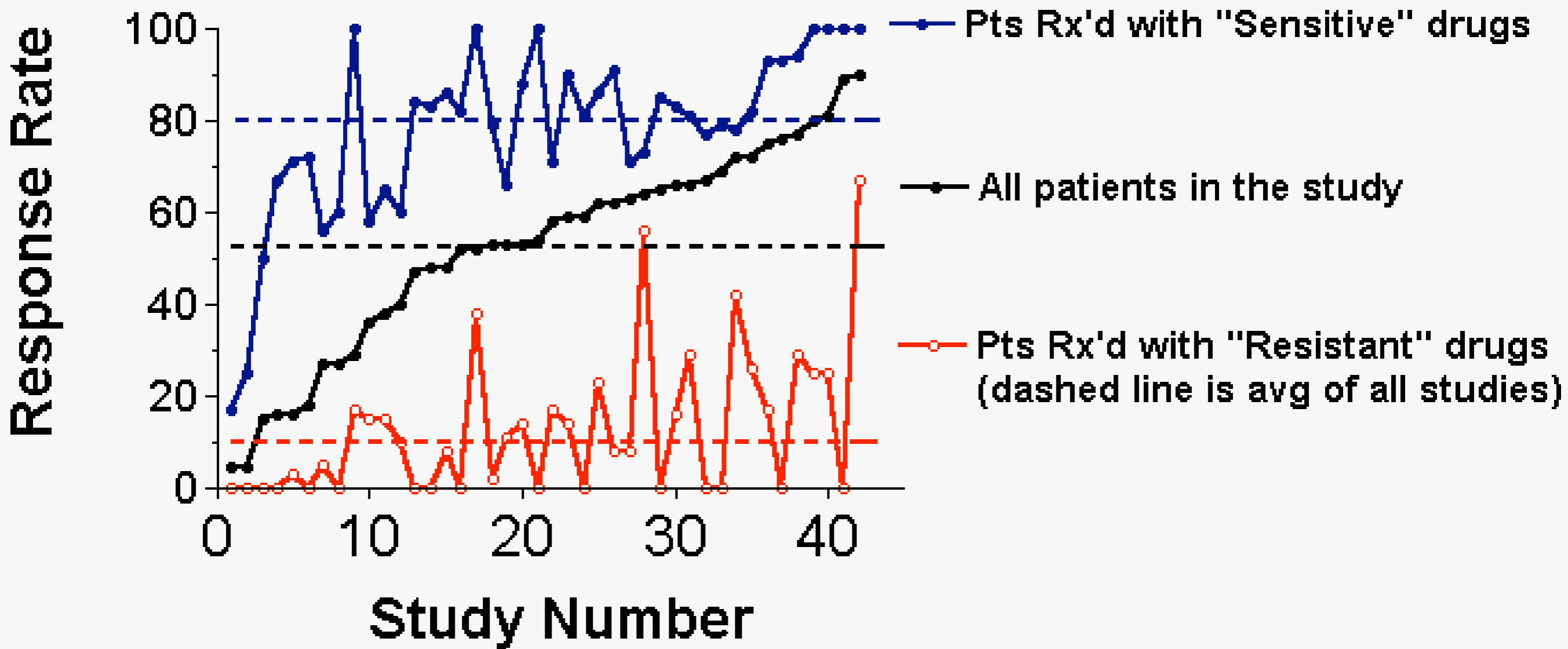
Tumor Cell Death, Expressed as Deviations from Mean of Reference Database Assays



T.S. prediction for 5FU in Colon Cancer



Correlation between LCA (cell death) results and clinical response to chemotherapy in 42 studies involving 1945 patients



Published Japanese Data

Kondo T, Kubota T, Tanimura T, Yamaue H, Akiyama S, Maehara Y, et al. Anticancer Res 20:2389, 2000

Table 2. Predictive values for chemosensitivity testing

No. of correlations attempted	S/S	S/R	R/S	R/R	Accuracy
1.101	215	246	45	595	74%

Overall response rate = 24%

Response rate "sensitive" = 47%

Response rate "resistant" = 7%

- There have been more than 25 peer review publications showing significant correlations between cell death assay results and patient survival

Journal of Clinical Oncology, Vol 22, 2004: pp. 3631-3638

American Society of Clinical Oncology Technology
Assessment: Chemotherapy Sensitivity and Resistance
Assays

Deborah Schrag, Harinder S. Garewal, Harold J. Burstein, David J. Samson,
Daniel D. Von Hoff, Mark R. Somerfield for the ASCO Working Group on
Chemotherapy Sensitivity and Resistance Assays

Methods:

**“We excluded reports that only reported correlations
between assay results and clinical outcomes.”**

...has metastatic colorectal cancer.
Now Genzyme can help you determine his
risk of serious adverse effects
before
he starts therapy.

Genzyme now offers the
Invader® UGT1A1 Molecular Assay,
an FDA-cleared innovative
screening test designed to
help you identify patients
who are at increased risk
for severe toxicity when
treated with irinotecan.
This simple blood test
will assist you in
making adjustments
in your patient's
therapy before
adverse effects
occur.



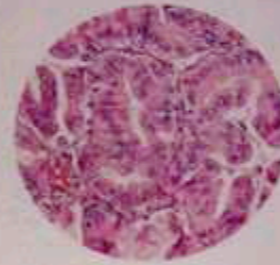
For more information about
Genzyme's cancer testing
services, including our
menu of innovative
tests that can help
physicians understand
a patient's response to
cancer therapy, visit
www.genzyme.com
or call (800) 467-0986.

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Innovative® (2012) Molecular Assay
is a registered trademark of Genzyme Corporation.
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Experience Tomorrow's Cancer Testing Laboratory Today.

genzyme

Why is it important to test for mutations in the EGFR gene?



Because choosing the most effective treatment is important for Nancy.

The future of cancer treatment lies in molecular-targeted therapy, the backbone of which is molecular diagnostic testing. Genzyme now offers EGFR mutation analysis, a molecular test for patients with non-small cell lung cancer.



Somatic mutations in the EGFR gene have been reported in ~85% of patients who respond to molecular-targeted therapies.

For more information about Genzyme's broad menu of cancer testing services, visit www.genzymegenetics.com or call (800) 447-5816.

Experience Tomorrow's Cancer Testing Laboratory Today.

genzyme

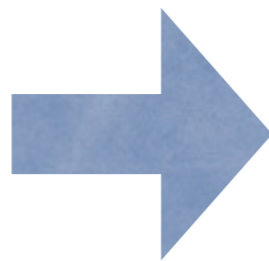
Circle No. 26 on reader service card

The "Bar" for Predictive Tests



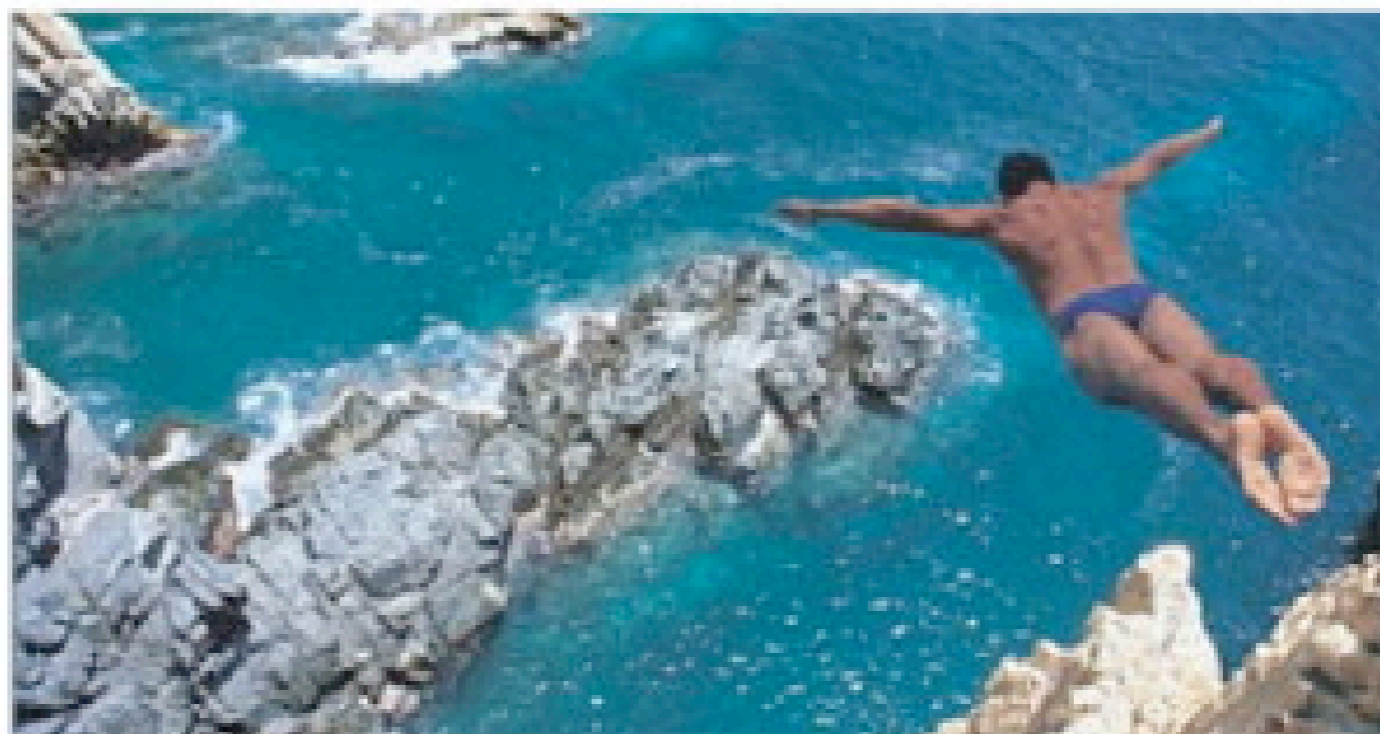
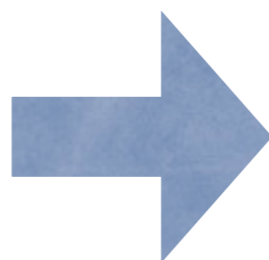
Standard for Genomic Tests

Standard for
ER, PR, Her2/Neu,
Panels of IHC
stains, EGFR
mutations,
OncotypeDx, etc.
etc.



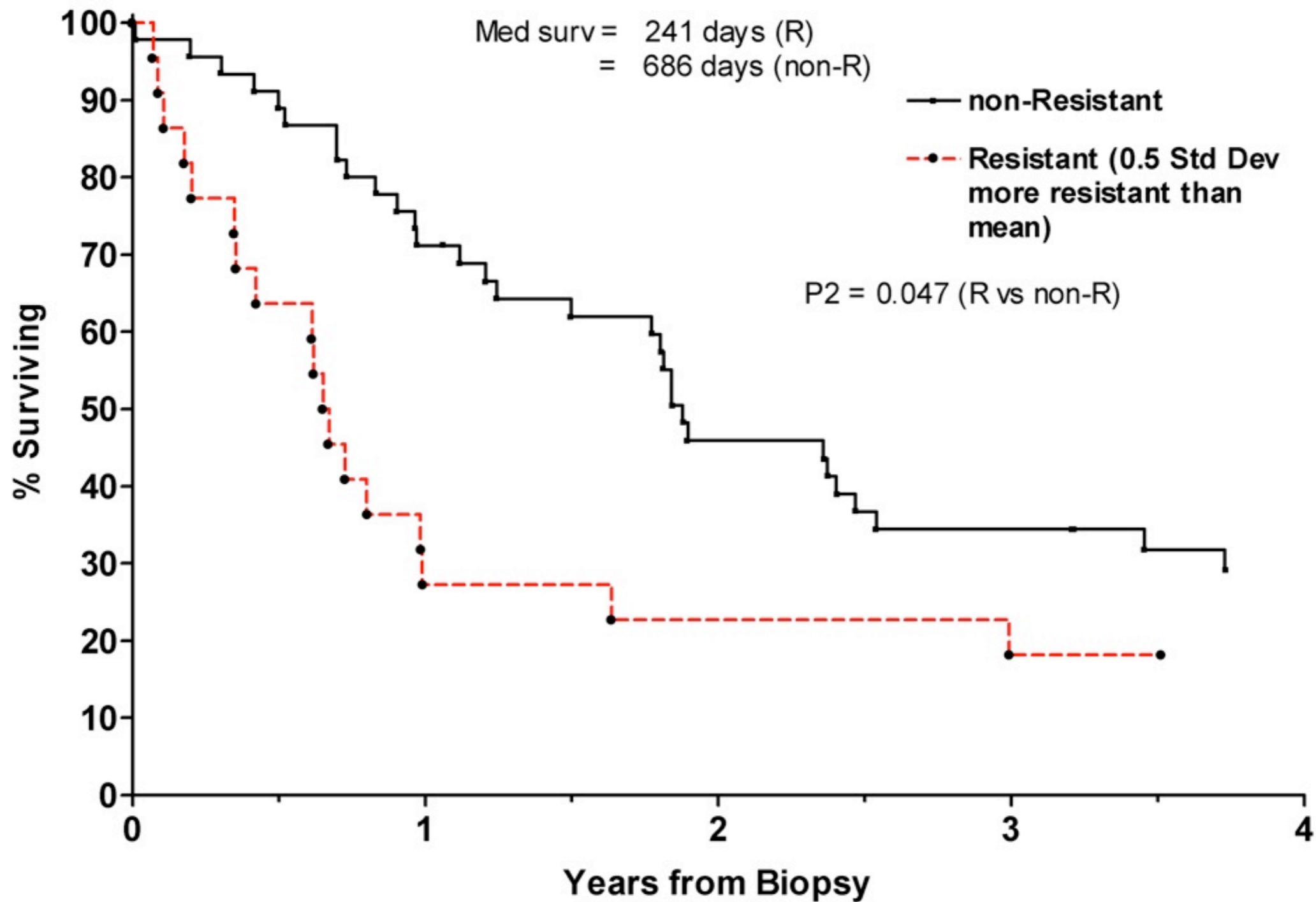
Standard for Cell Culture Tests

Standard for
Cell culture assays

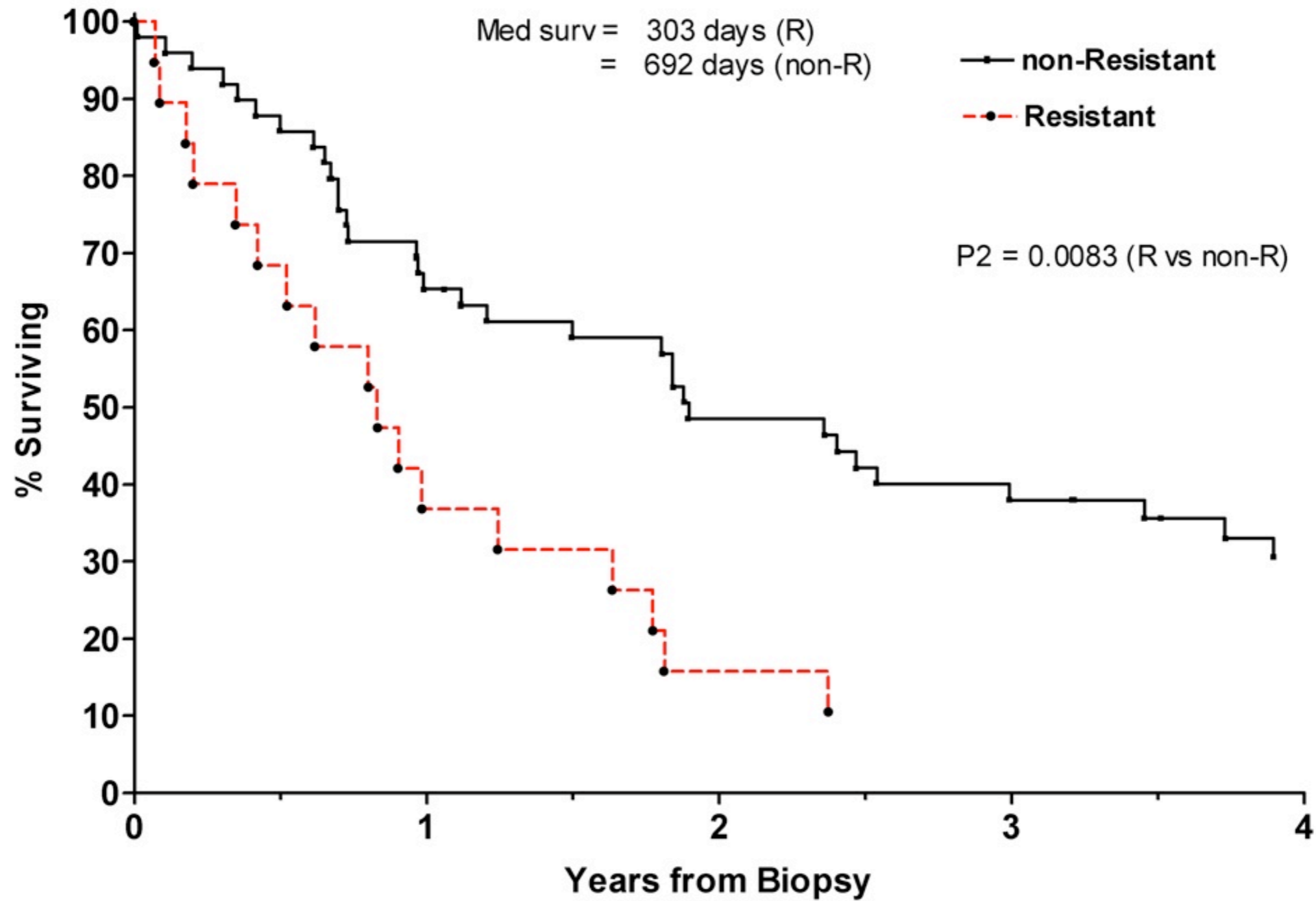


Prospectively reported cell death (MTT,
DISC, resazurin) assay results and patient
survival in **stage 4 colon cancer**
(Weisenthal, unpublished)

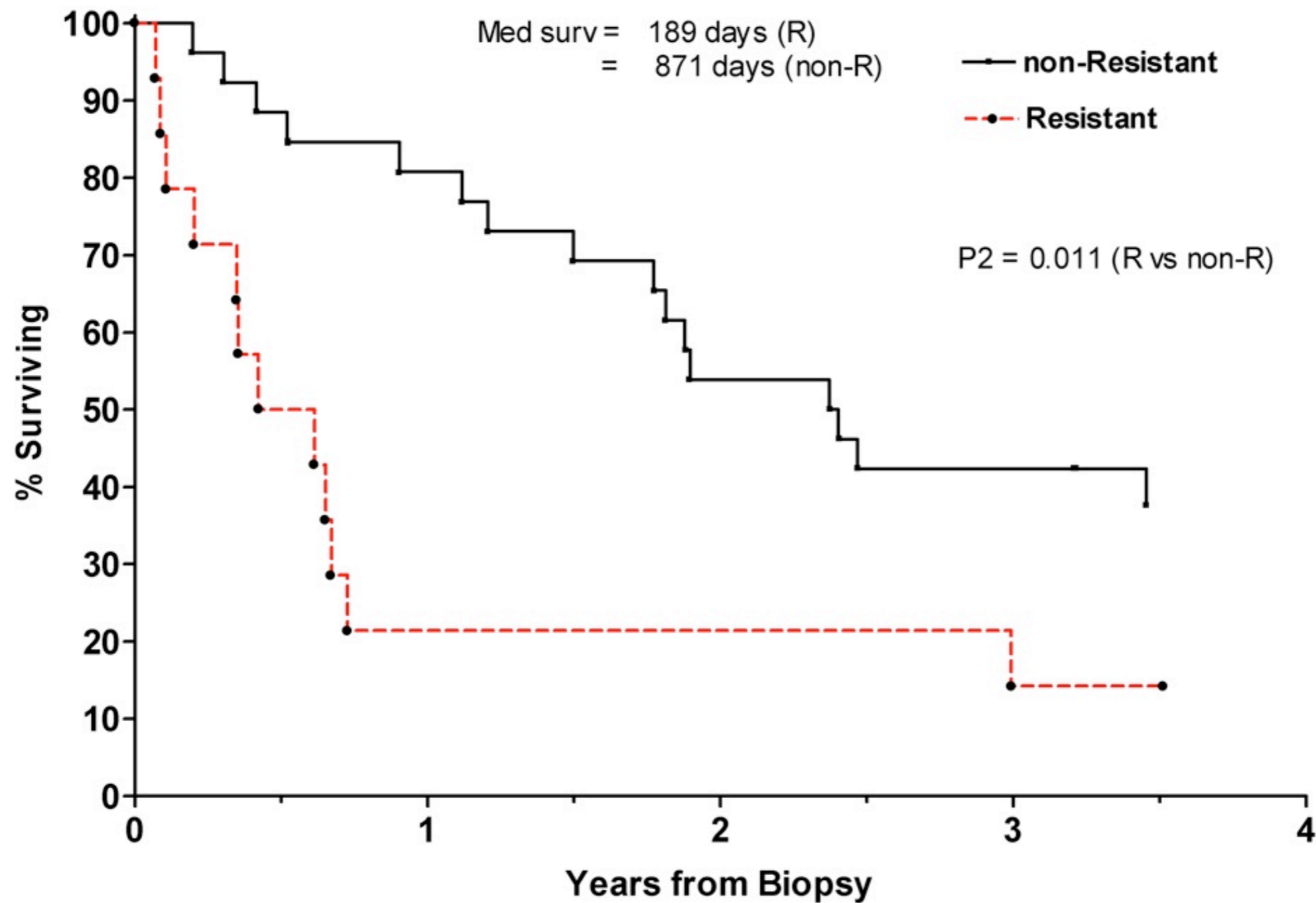
**Stage IV Colon Cancer
Previously-Untreated
Survival as a function
of 5FU activity in vitro
(MTT Assay; 40 ug/ml; 96 hrs)
Stage 4**



Stage IV Colon Cancer
Previously-Untreated
Survival as a function
of 5FU activity in vitro
(MTT Assay; 20 ug/ml; 96 hrs)
Stage 4

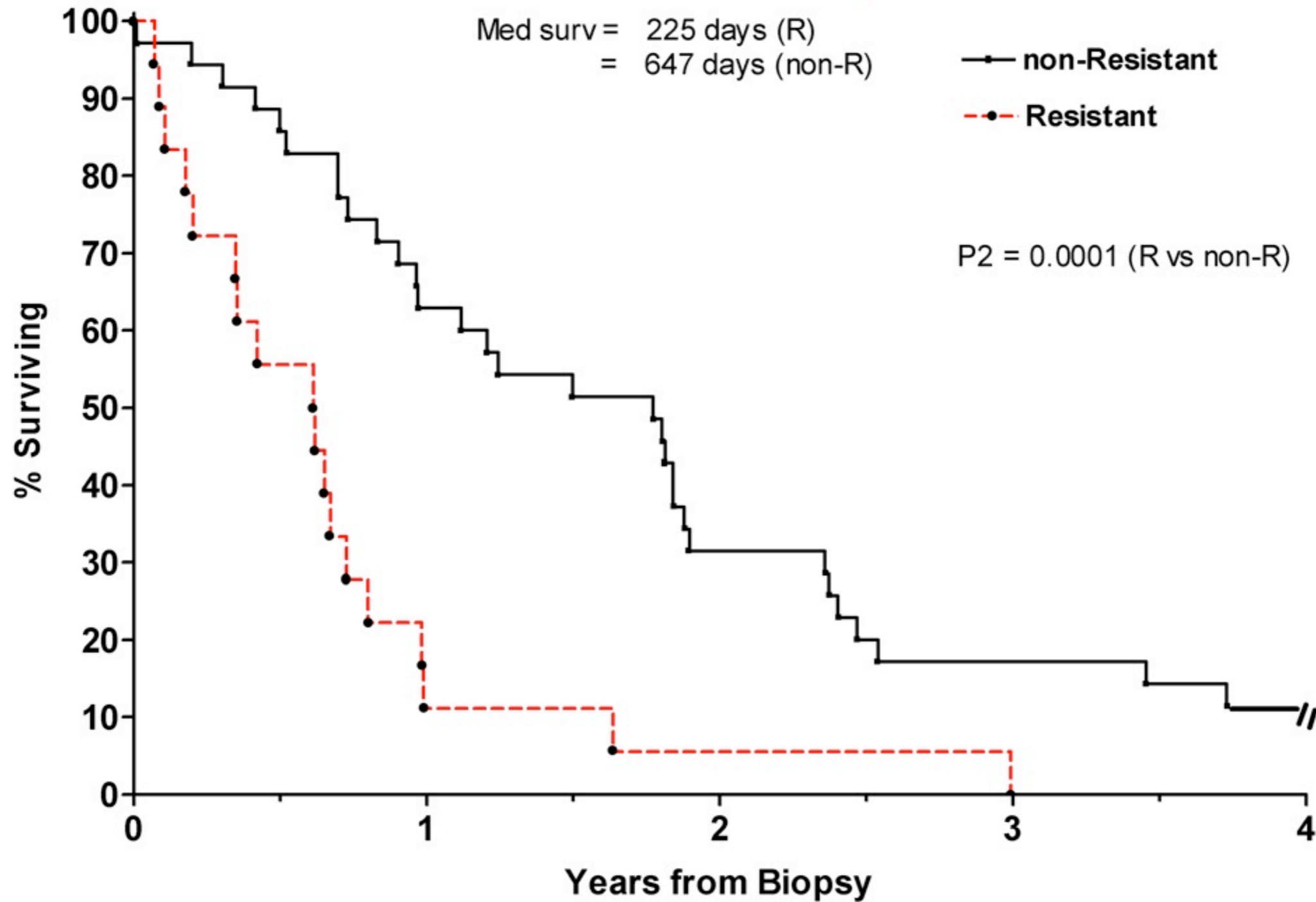


Stage IV Colon Cancer
 Previously-Untreated
 Survival as a function
 of 5FU activity in vitro
 (MTT Assay; 40 ug/ml; 96 hrs)
 Stage 4; Both DISC/MTT Evaluable

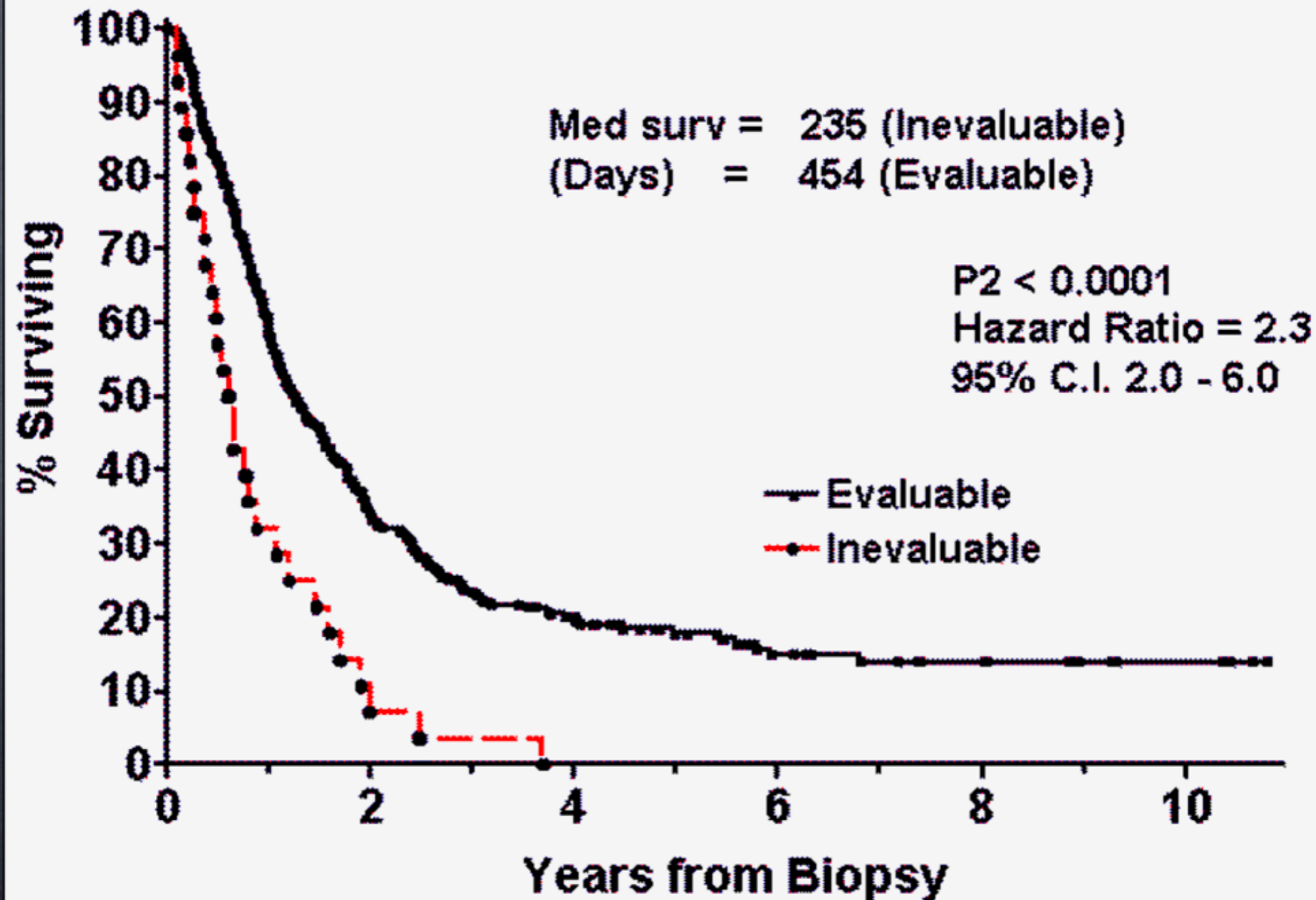


Stage IV Colon Cancer
Previously-Untreated
Survival as a function
of 5FU activity in vitro
(MTT Assay; 40 ug/ml; 96 hrs)
Stage 4

Dead Patients Only



Colon Cancer
All Extracolonic Biopsy Sites
Survival as a Function of Whether
Cell Culture Drug Resistance
Testing Was Evaluable or Inevaluable
(Minimum 3 year follow up)



Cell Death Assays (CDAs), continued

- CDAs show disease-specific drug activity
- CDAs are useful clinical and research tools for “targeted” drugs, examples: Gefitinib and Bevacizumab
- CDAs provide unique information complementary to that provided by “molecular” tests

• The “Holy Grail” of preclinical drug development is the identification of **DISEASE-SPECIFIC** drug activity.

US National Cancer Institute "disease oriented" 60 human tumor cell line drug evaluation system

**Display and Analysis of
Patterns of Differential Activity
of Drugs Against Human
Tumor Cell Lines: Development
of Mean Graph and
COMPARE Algorithm**

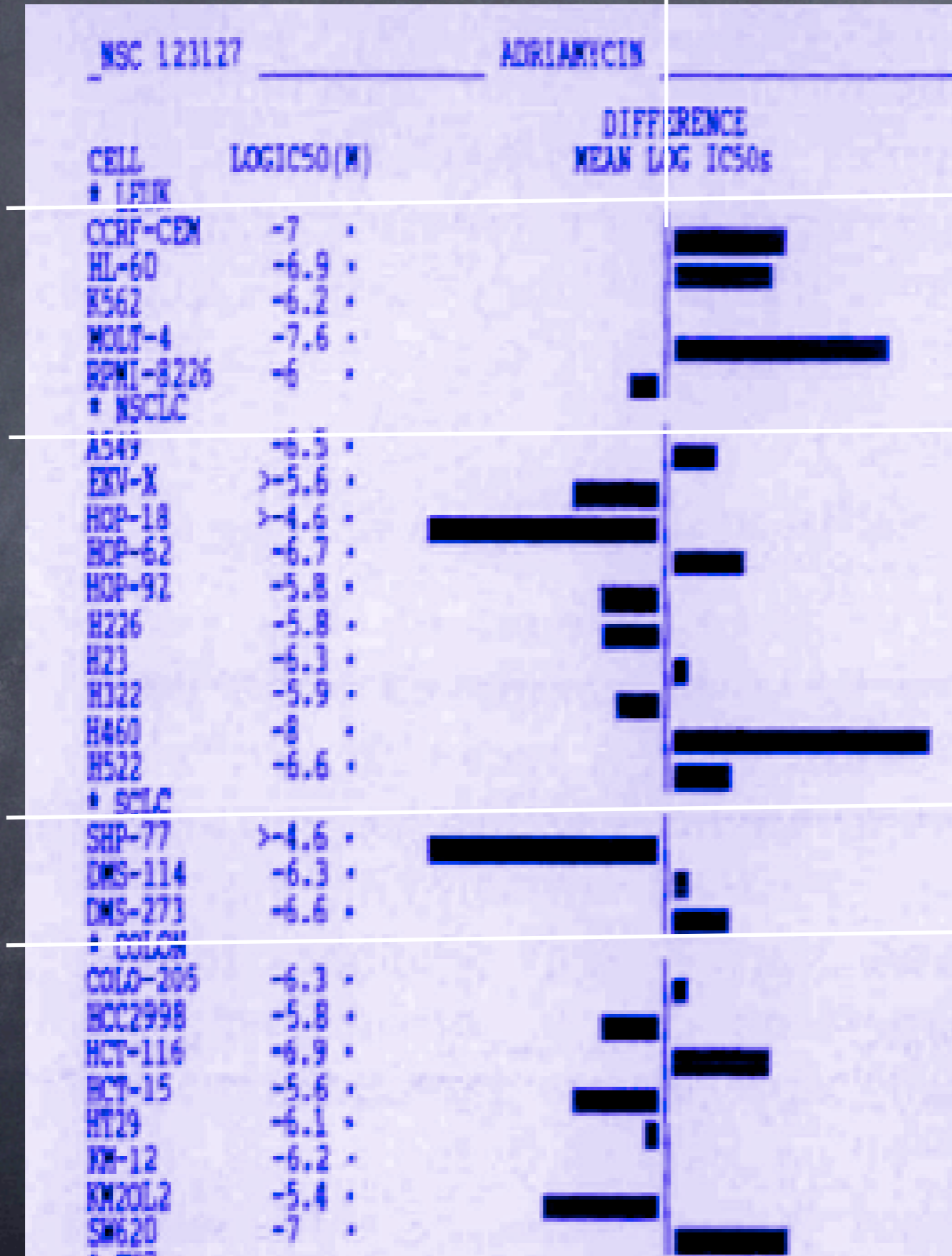
K. D. Paull, R. H. Shoemaker,
L. Hodes, A. Monks,
D. A. Scudiero, L. Rubinstein,
J. Plowman, M. R. Boyd*

Journal of the National Cancer Institute
81:1088, 1989

←--More "Resistant"

More "Sensitive"-->

Doxorubicin
(Adriamycin)



Leukemia

NSCLC

SCLC

Colon

Established cell lines

JNCI 81:1088,'89

Disease Specific Activity: Fresh tumor cell death assays

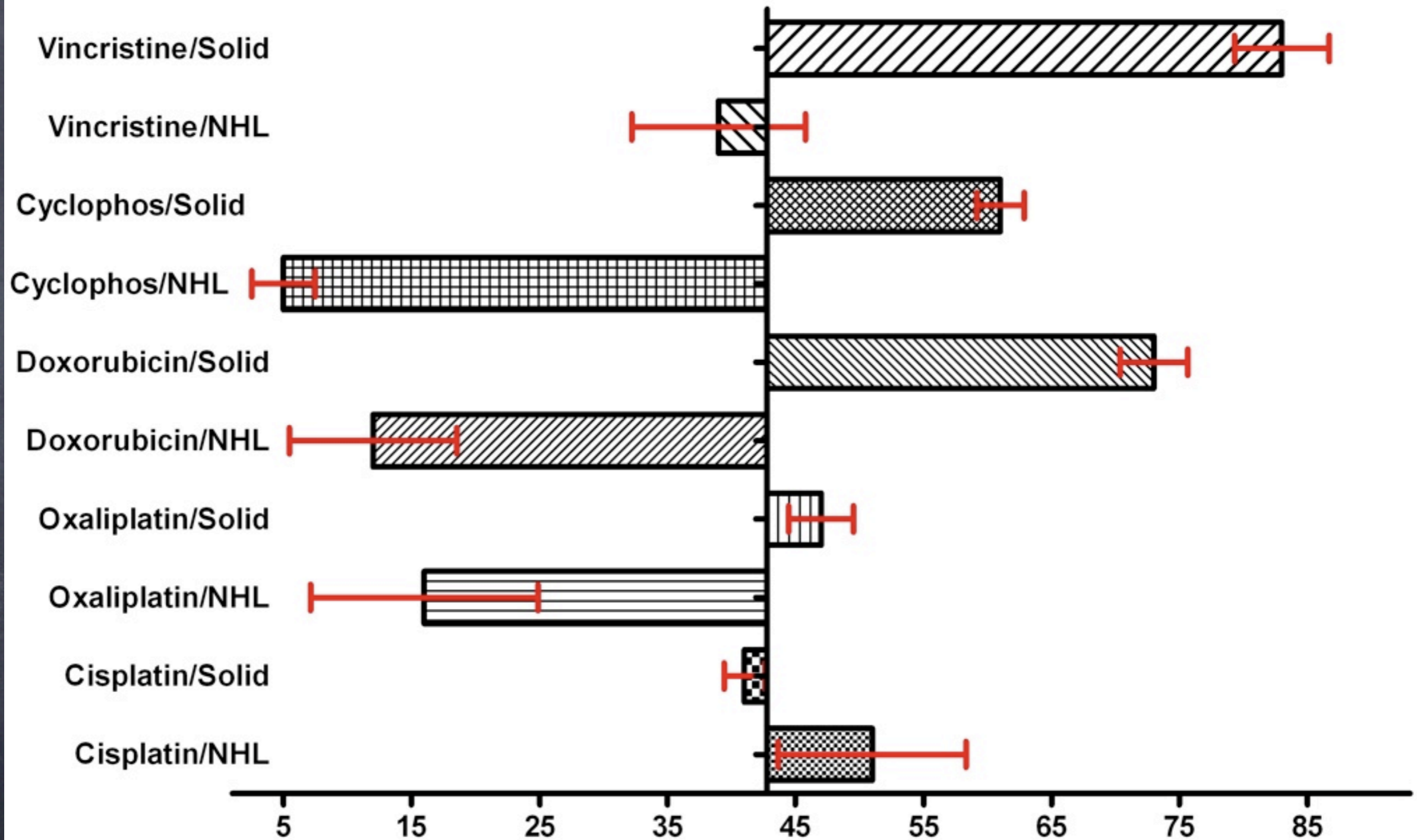
		Comparing				
Drug Tested		Cisplatin		Cisplatin		
Clinical Diag		OVARIAN		COLON		
Prior Treat.		No		No		
DISC	Mean	Hi	Lo	Hi	Lo	
	Freq	25	52	48	76	
	StdDev	26	29	25	16	
		n =	212	210	127	127
MIT	Mean	Hi	Lo	Hi	Lo	
	Freq	26	53	49	76	
	StdDev	22	27	20	18	
		n =	212	210	151	152

Values are mean percent control cell survival

Disease-Specific Activity: Lymphoma vs. Solid Tumors

<-- More "Sensitive"

More "Resistant" -->



Mean % Control Cell Survival

Red Lines 95% Confidence Intervals

Doxorubicin

<-- More sensitive

More resistant -->

Carcinoid

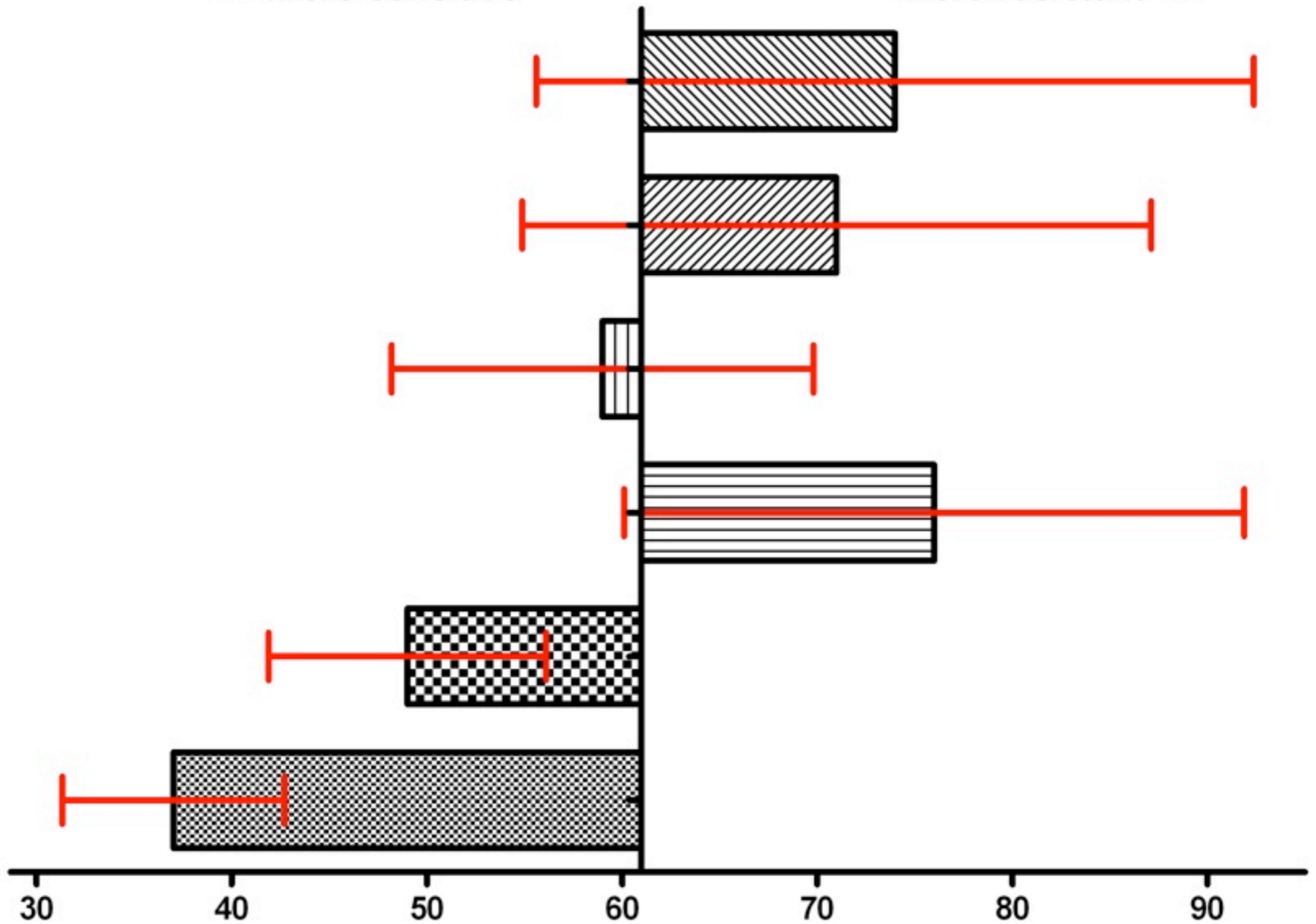
Renal Cell

Lung Adeno

Colon

Ovarian

Breast



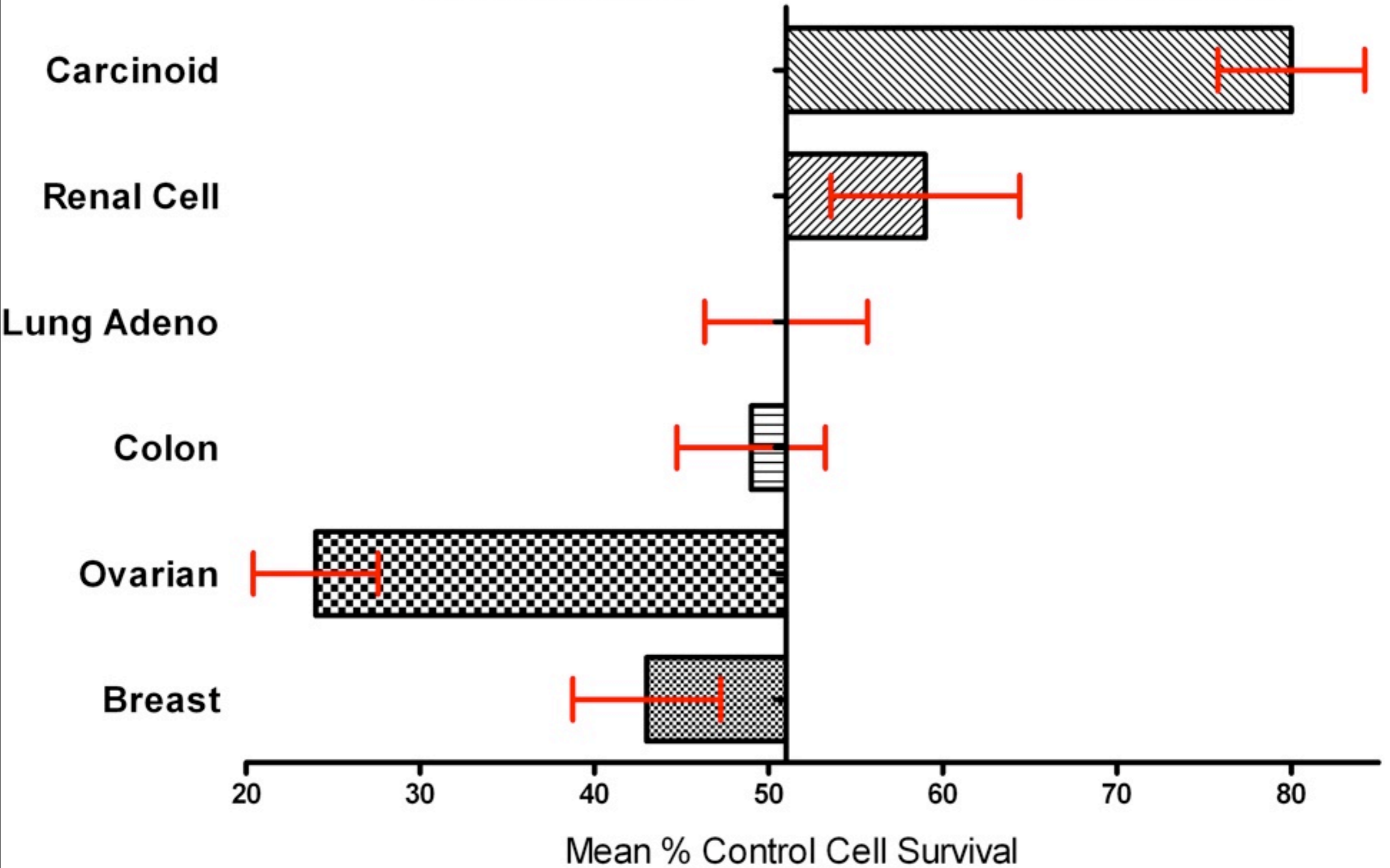
Mean % Control Cell Survival

Red Bars 95% Confidence Intervals

Cisplatin

<-- More sensitive

More resistant -->



Red Bars 95% Confidence Intervals

Oxaliplatin

<-- More sensitive

More resistant -->

Carcinoid

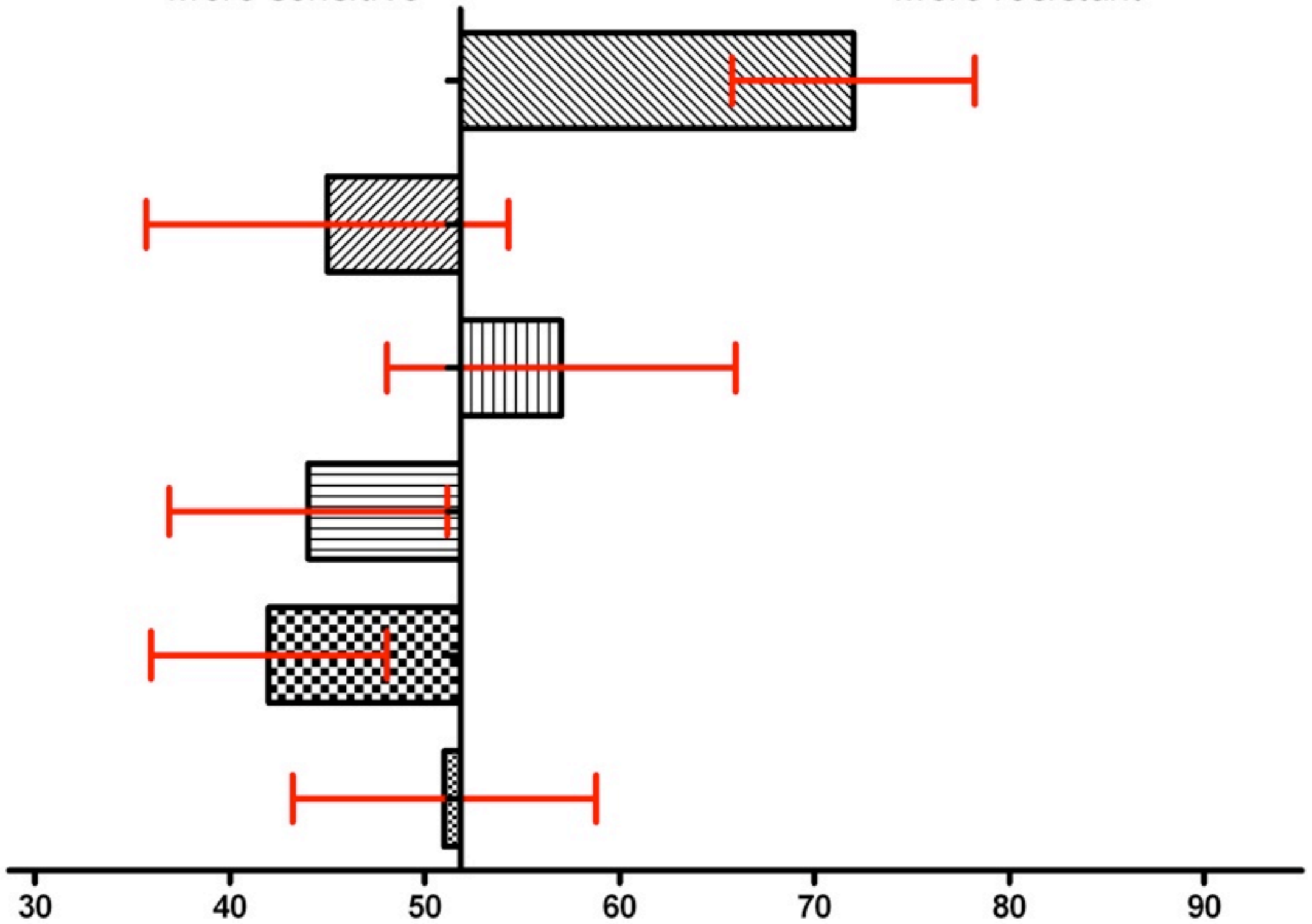
Renal Cell

Lung Adeno

Colon

Ovarian

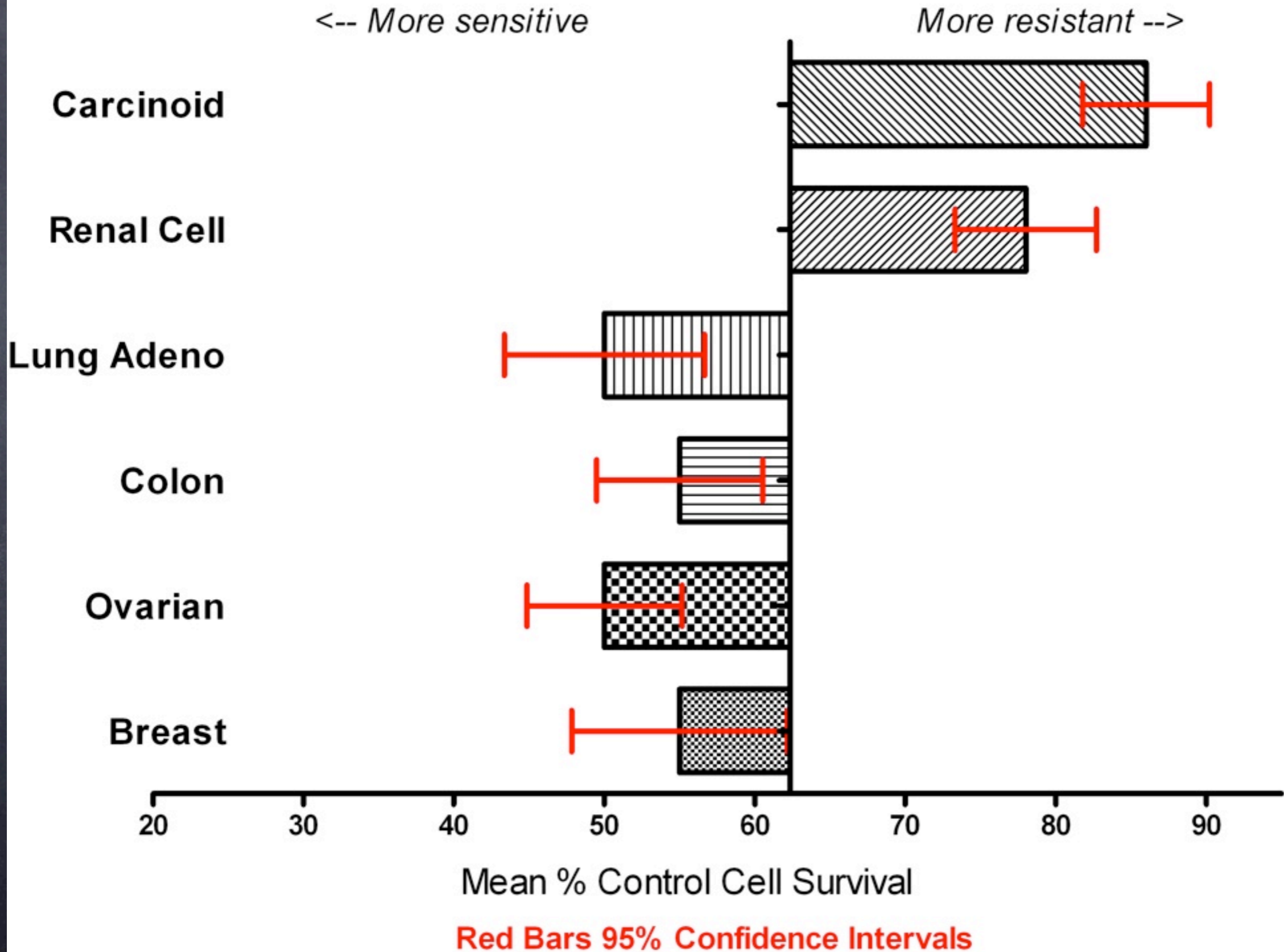
Breast



Mean % Control Cell Survival

Red Bars 95% Confidence Intervals

Irinotecan



Topotecan

<-- More sensitive

More resistant -->

Carcinoid

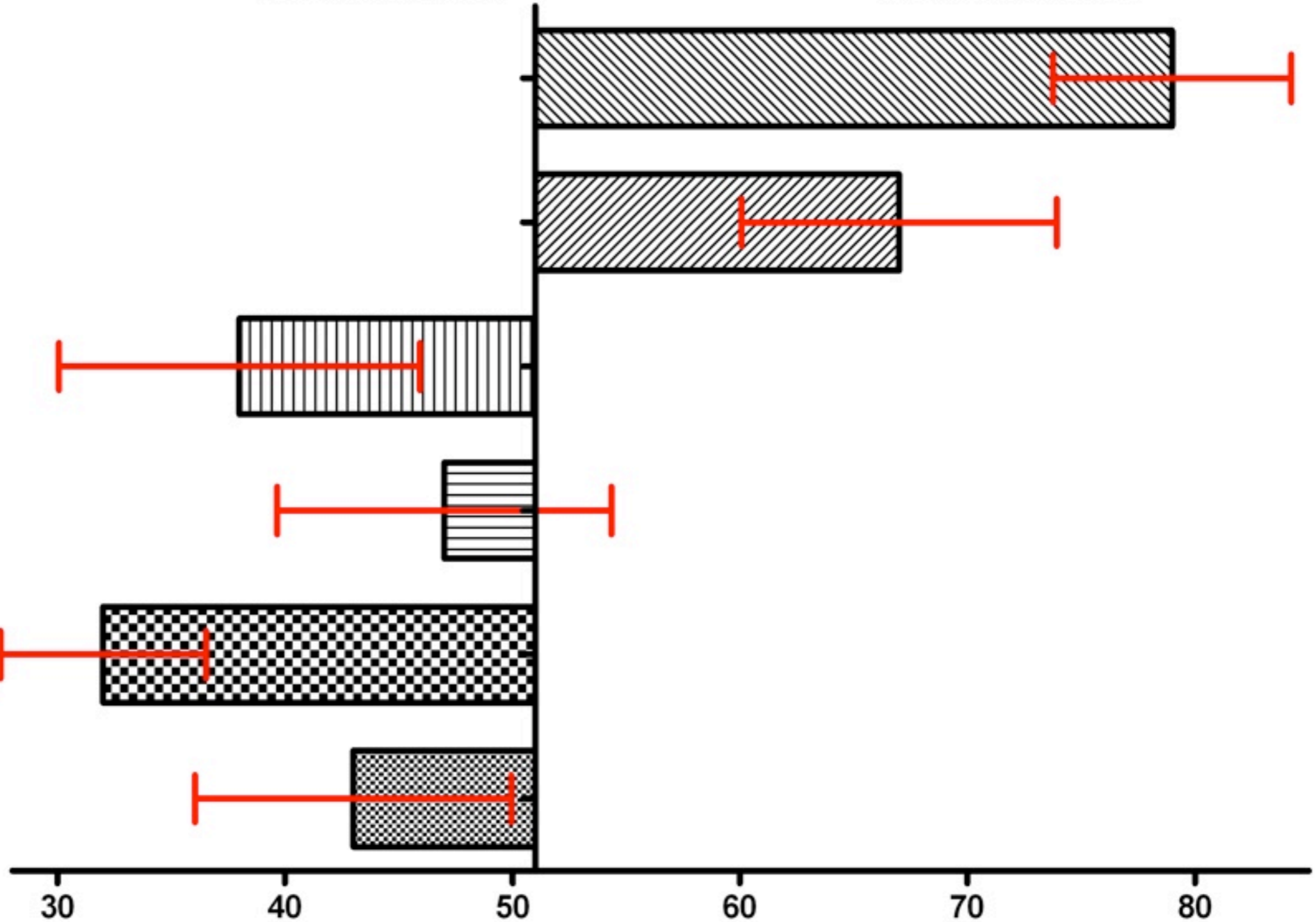
Renal Cell

Lung Adeno

Colon

Ovarian

Breast



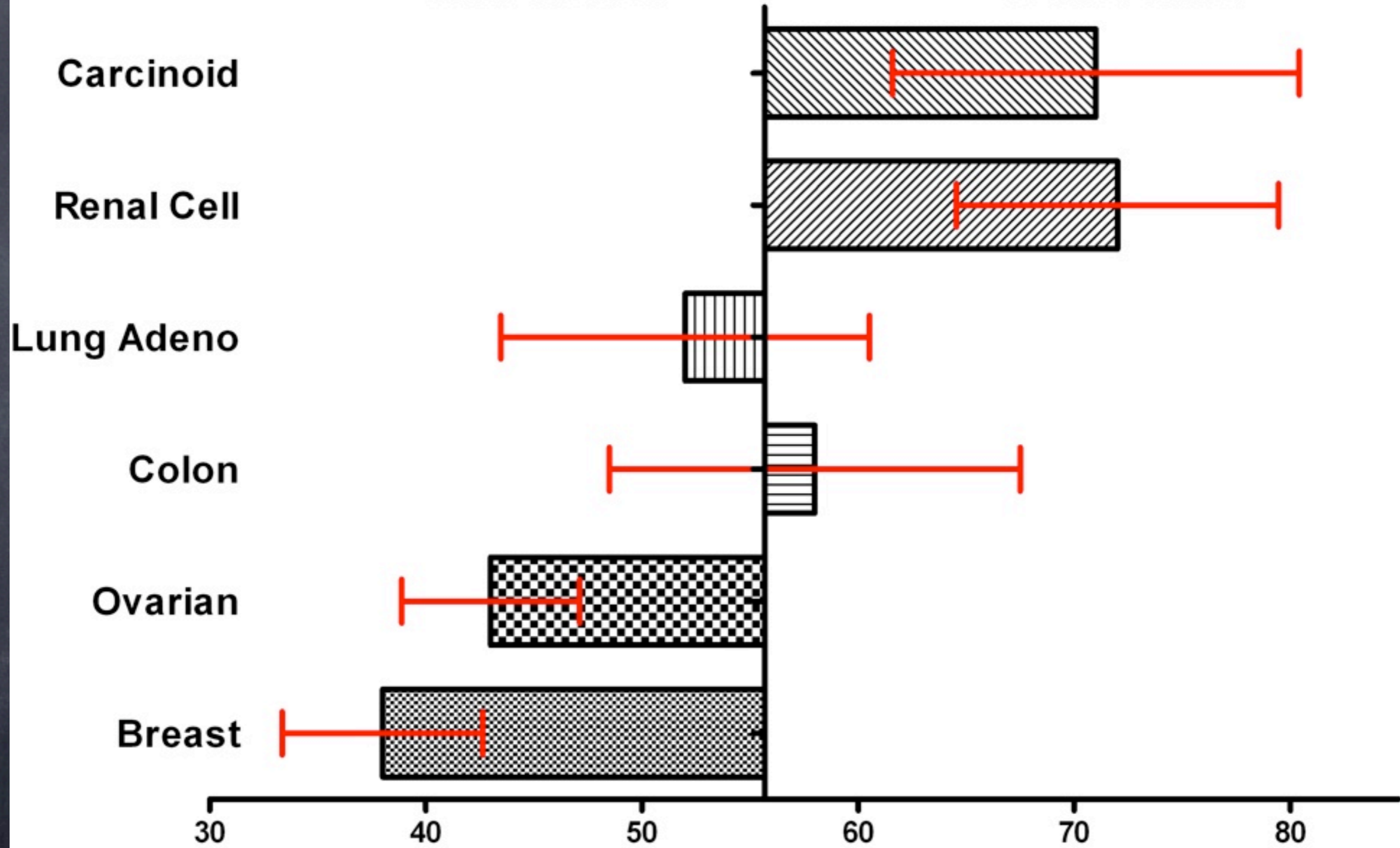
Mean % Control Cell Survival

Red Bars 95% Confidence Intervals

Melphalan

<-- More sensitive

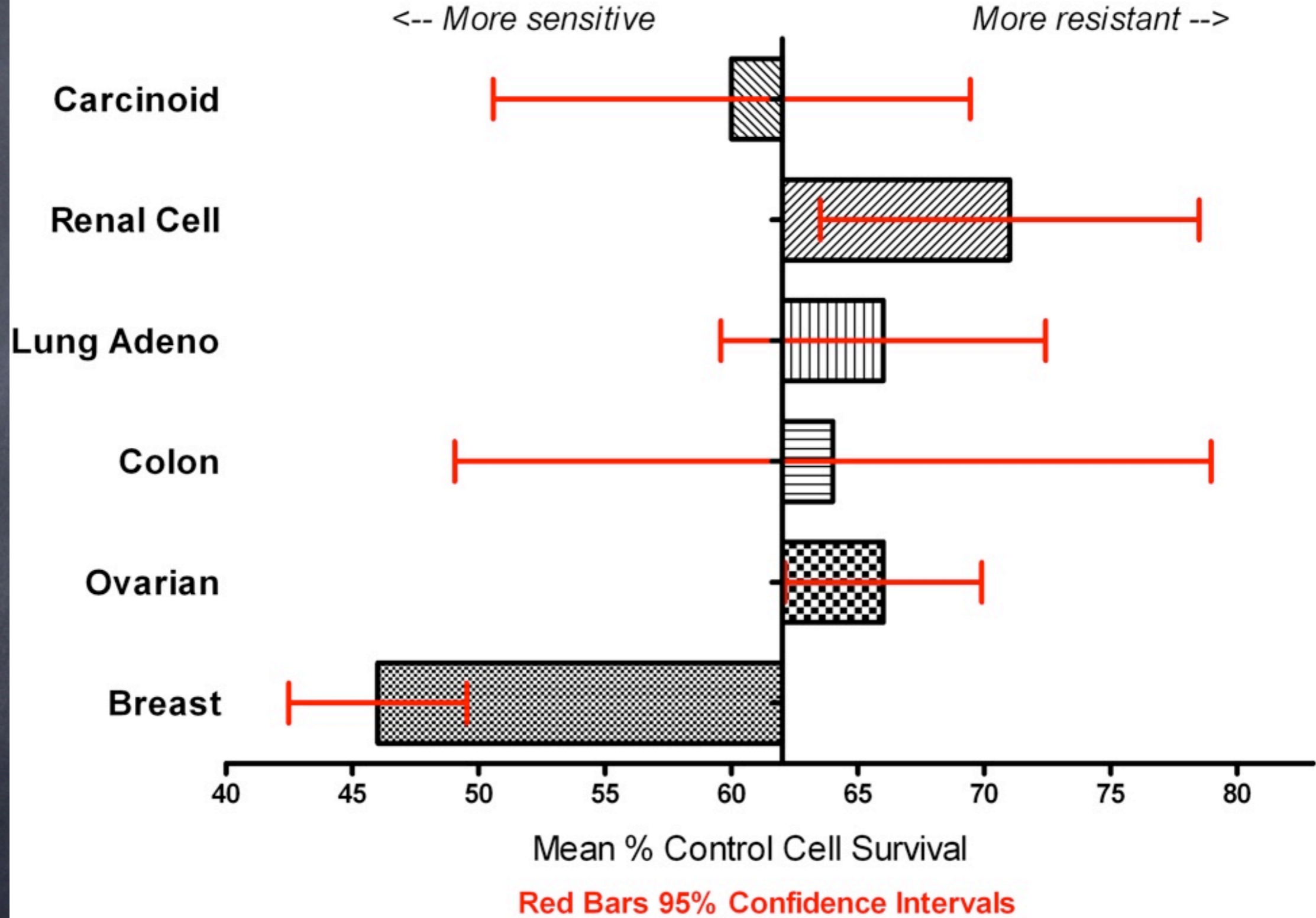
More resistant -->



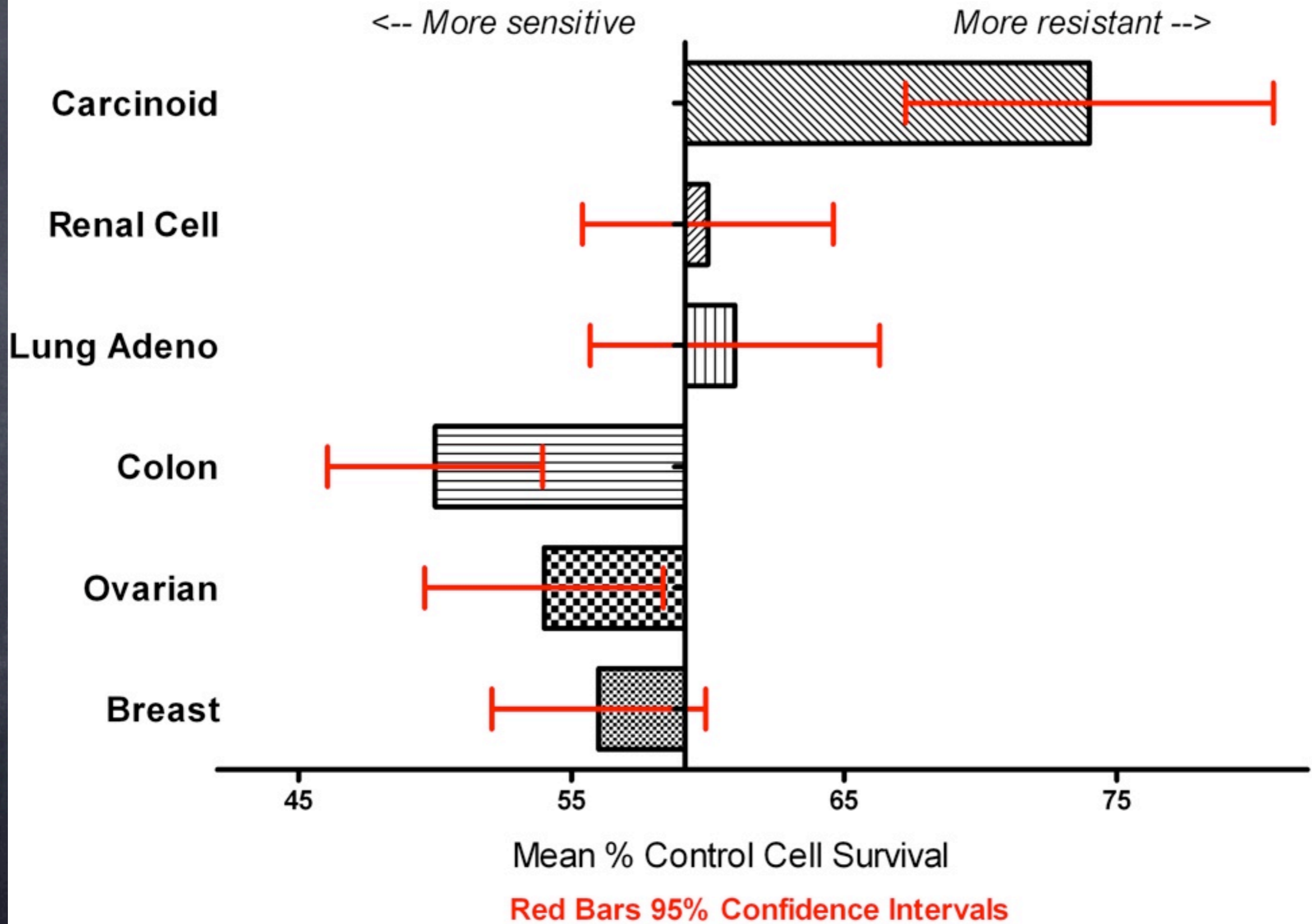
Mean % Control Cell Survival

Red Bars 95% Confidence Intervals

Cyclophosphamide (4HC)



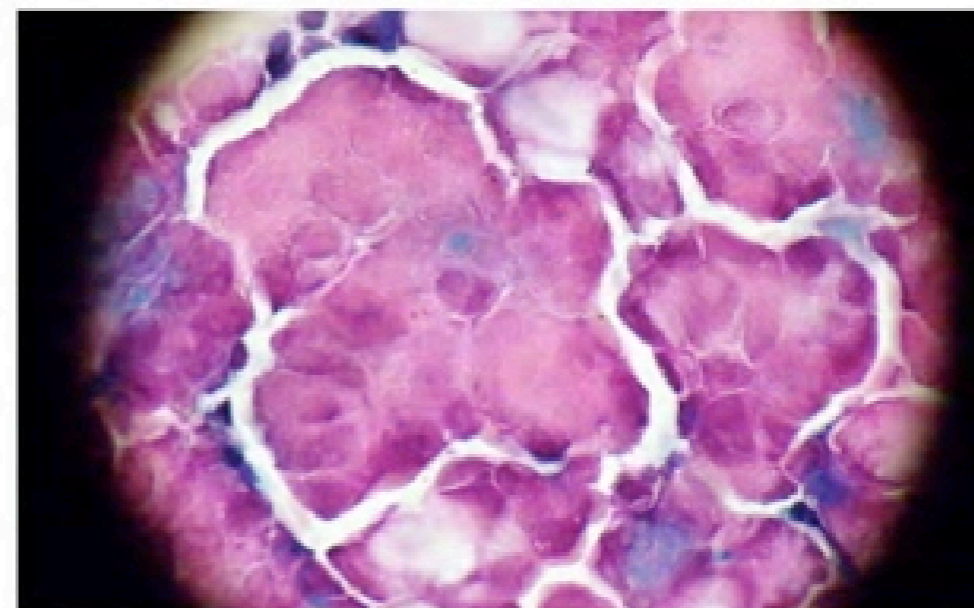
5-Fluorouracil



Specimen Information

Collected: 08/29/06 Path. Accession No.: RS-06-10467
 Received: 08/30/06 Specimen Site: Bowel/Ileum
 Reported: 09/08/06

Specimen
Quality
Factors



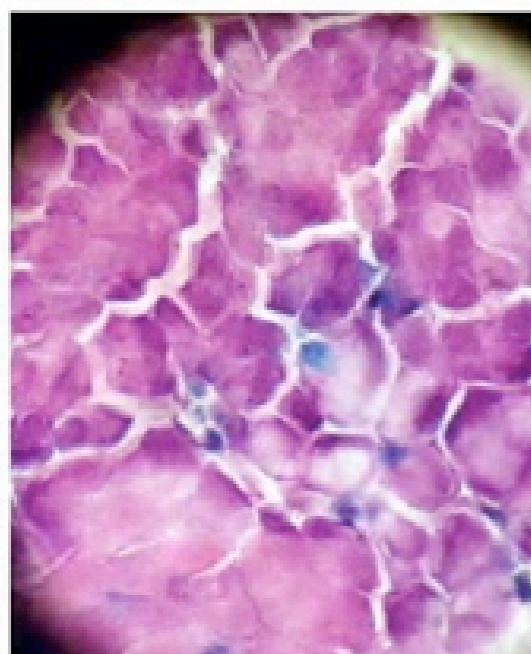
Control Culture

EGFR[™] Assay Detail

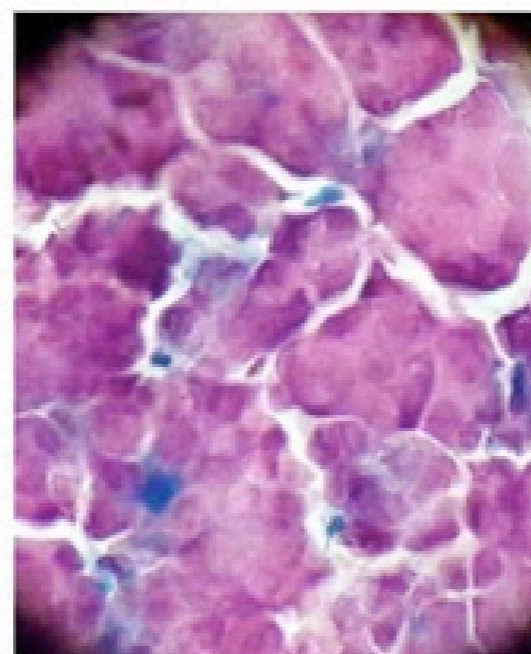
Assay/Analysis Name: EGFRx
 Analysis Type: Whole Cell Profiling
 Endpoint: Cell morphology plus cell metabolism
 Agent Class: Kinase Inhibitor
 Pathway/Mechanism: EGFR/ Kinase Signaling

EGFR[™] Assay - Cellular Response Profiles

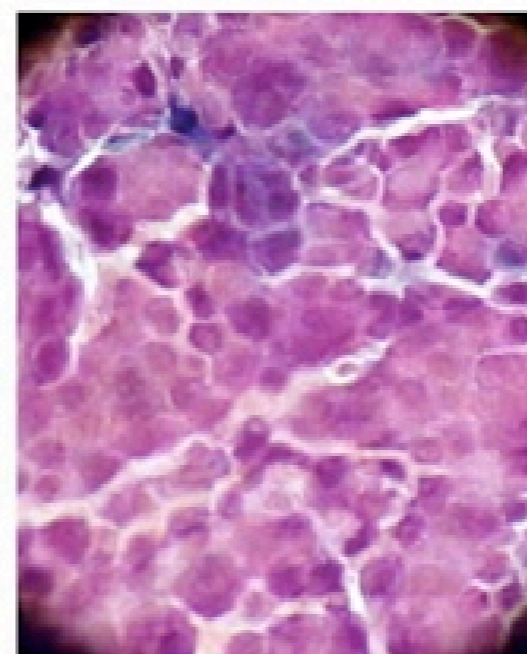
Targeted Therapy Agent	Drug Activity	Activity Category
Erlotinib (Tarceva)	Low	Unfavorable
Gefitinib (Iressa)	Low	Unfavorable
Sunitinib (Sutent)	Low	Unfavorable



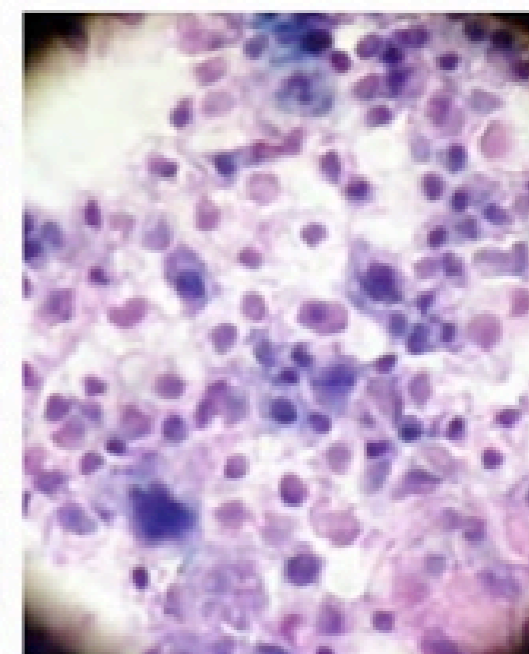
Erlotinib



Gefitinib



Sunitinib

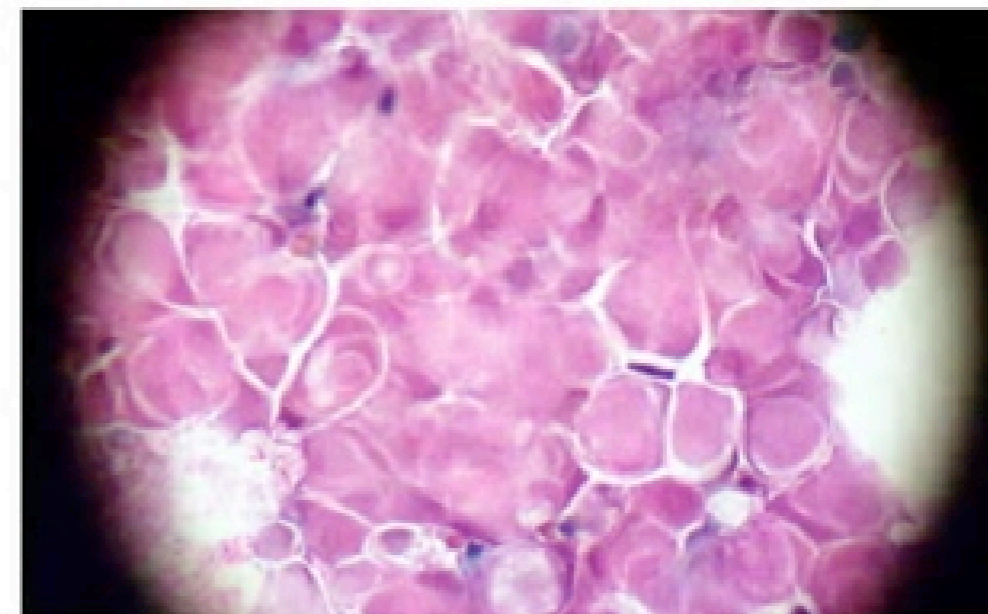


Sorafenib

Specimen Information

Collected: 09/21/06 Path. Accession No.: 06-9280
Received: 09/22/06 Specimen Site: Scalp&Liver
Reported: 10/04/06

Specimen
Quality
Factors



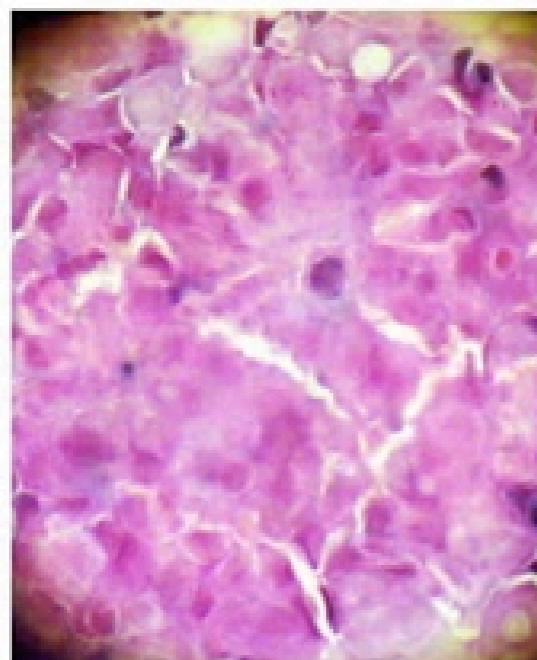
Control Culture

EGFRx™ Assay Detail

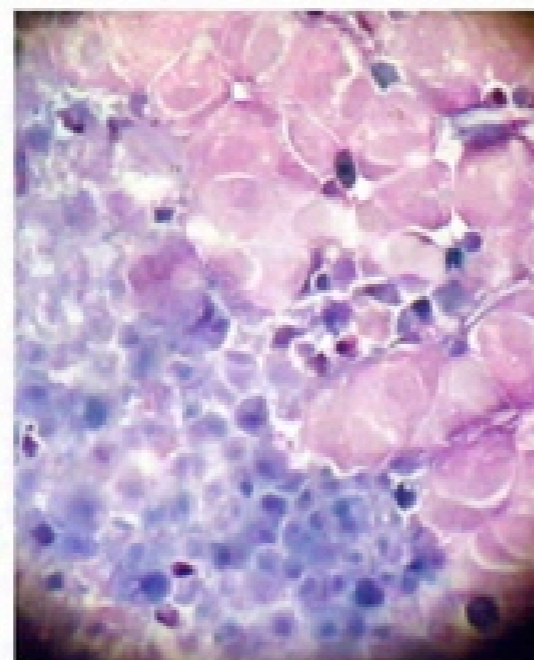
Assay/Analysis Name: EGFRx
Analysis Type: Functional Profiling
Endpoint: Cell Metabolism + Cell Morphology
Agent Class: Kinase Inhibitor
Pathway/Mechanism: EGFR/Kinase Signaling

EGFRx™ Assay - Cellular Response Profiles

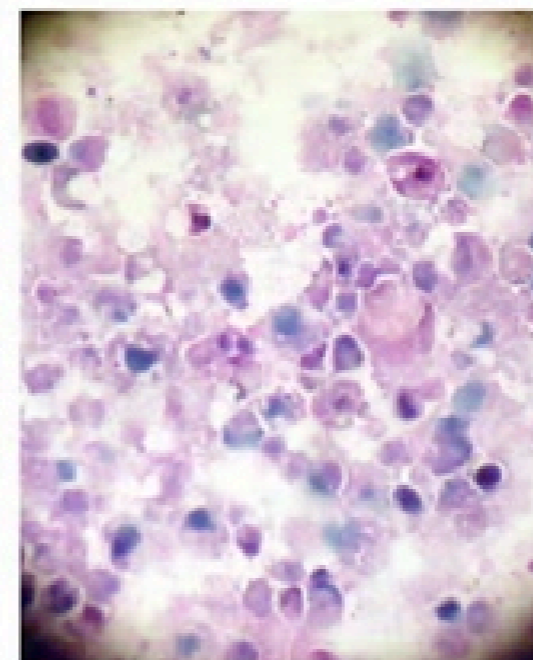
Targeted Therapy Agent	Drug Activity	Activity Category
Erlotinib (Tarceva)	Low	Unfavorable
Gefitinib (Iressa)	Moderate	Borderline
Sorafenib	Moderate	Borderline
Sunitinib (Sutent)	High	Favorable



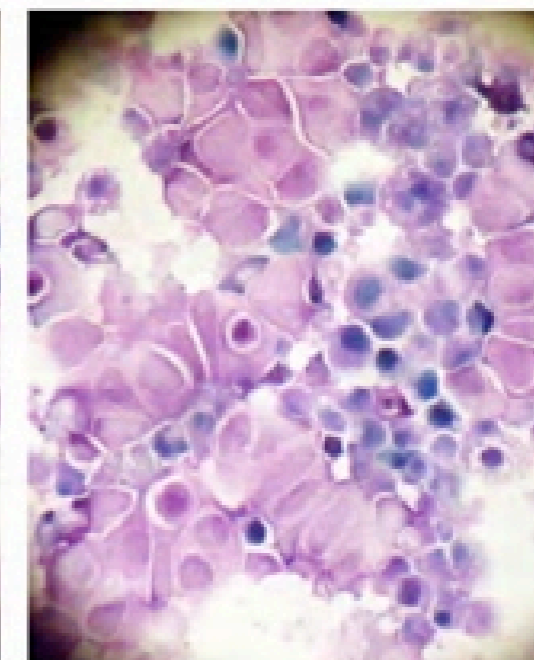
Erlotinib



Gefitinib



Sunitinib



Sorafenib

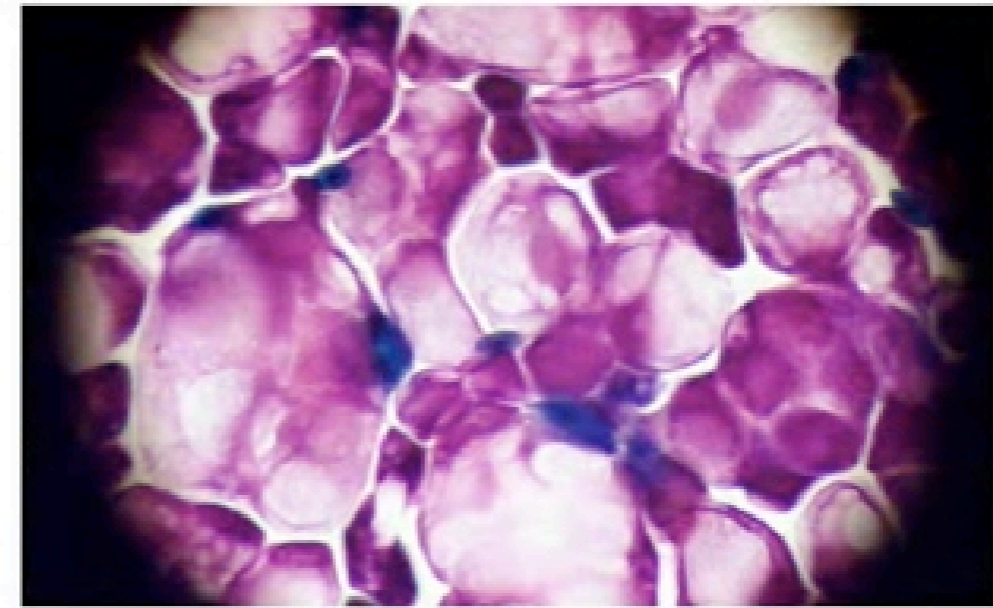
Specimen Information

Collected: 09/22/06 Path. Accession No.: HCC-06-1645
 Received: 09/23/06 Specimen Site: Fluid, Ascites
 Reported: 09/29/06

Specimen Quality Factors See assay description and "non-targeted" assay report.

EGFRTM Assay Detail

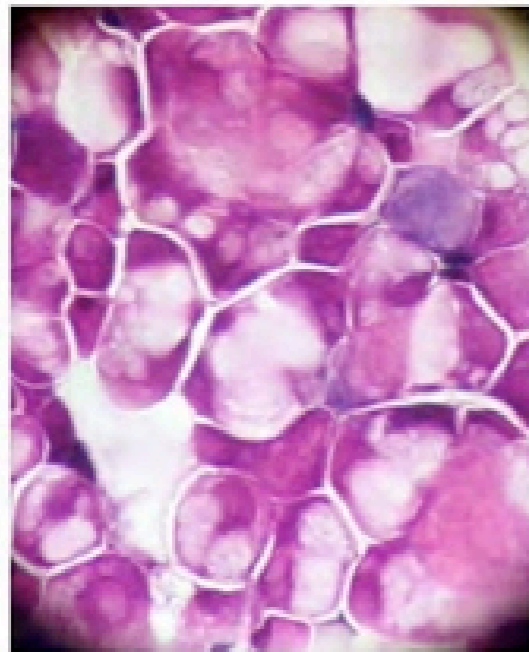
Assay/Analysis Name: EGFRx
 Analysis Type: Functional Cell Profiling
 Endpoint: Cell Metabolism/Cell Morphology
 Agent Class: Kinase Inhibitors
 Pathway/Mechanism: EGFR/Kinase Signaling



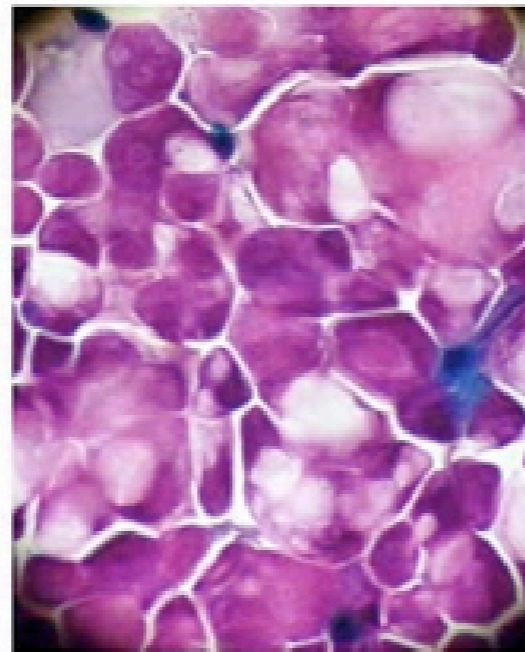
Control Culture

EGFRTM Assay - Cellular Response Profiles

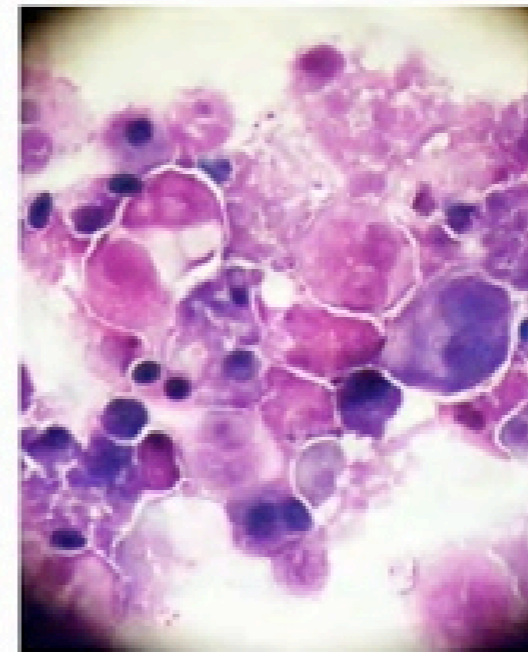
Targeted Therapy Agent	Drug Activity	Activity Category
Erlotinib (Tarceva)	Low	Unfavorable
Gefitinib (Iressa)	Low	Unfavorable
Sunitinib (Sutent)	Moderate	Borderline
Sorafenib (Nexavar)	High	Favorable



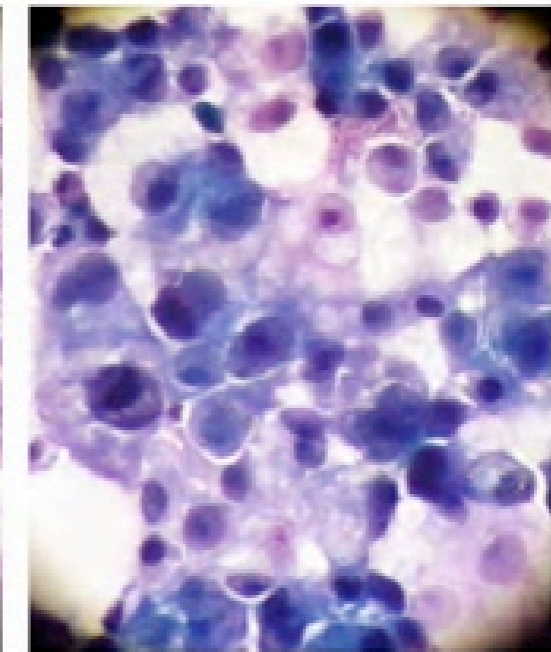
Erlotinib



Gefitinib



Sunitinib



Sorafenib

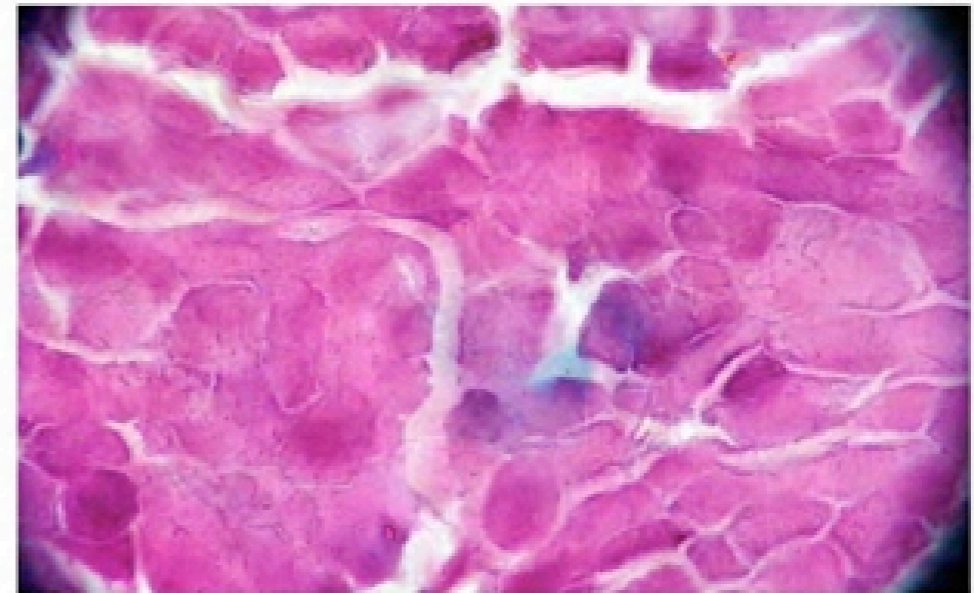
Specimen Information

Collected: 10/10/06 Path. Accession No.: TS-06-11382
 Received: 10/11/06 Specimen Site: Fluid,Pleural
 Reported: 10/20/06

Specimen Quality Factors Please see enclosed assay description and also "non-targeted" assay report.

EGFR[™] Assay Detail

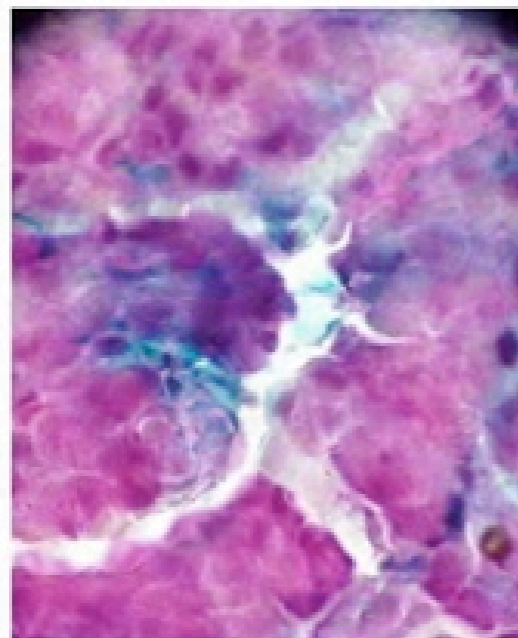
Assay/Analysis Name: EGFRx
 Analysis Type: Functional Profiling
 Endpoint: Cell Metabolism + Cell Morphology
 Agent Class: Kinase Inhibitor
 Pathway/Mechanism: EGFR/Kinase Signaling



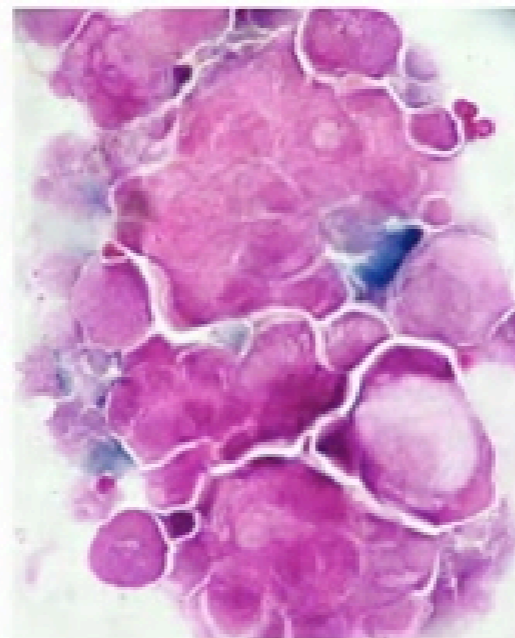
Control Culture

EGFR[™] Assay - Cellular Response Profiles

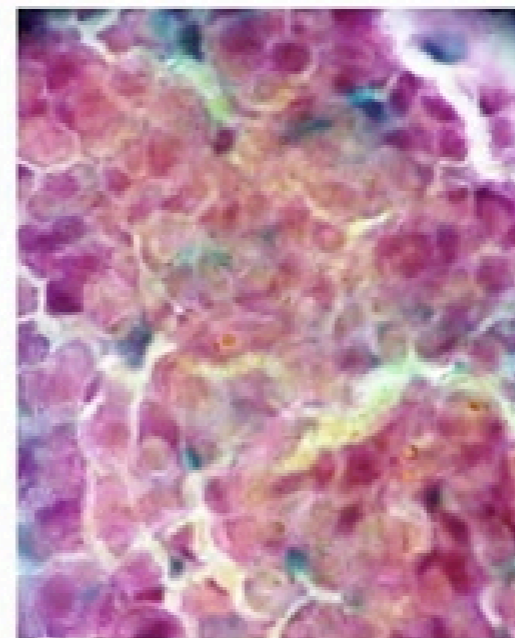
Targeted Therapy Agent	Drug Activity	Activity Category
Erlotinib (Tarceva)	Low	Unfavorable
Gefitinib (Iressa)	Low	Unfavorable
Sorafenib	Low	Unfavorable
Sunitinib (Sutent)	Low	Unfavorable



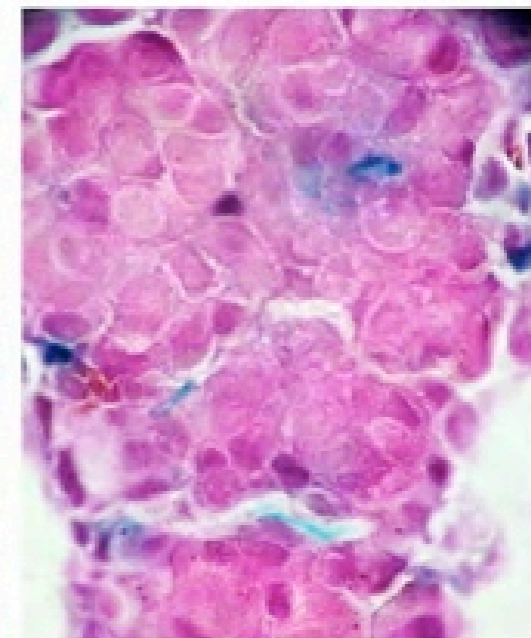
Erlotinib



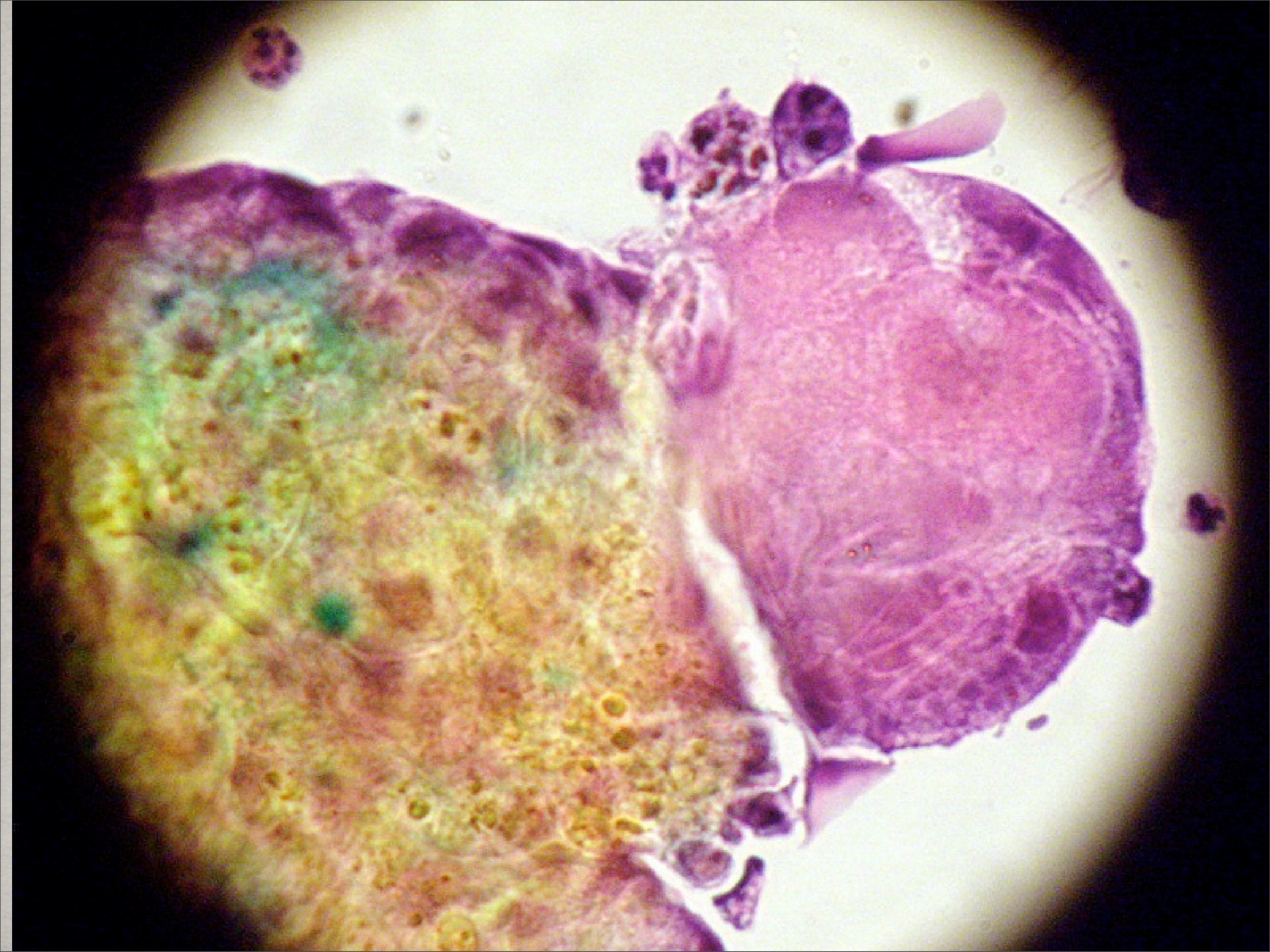
Gefitinib

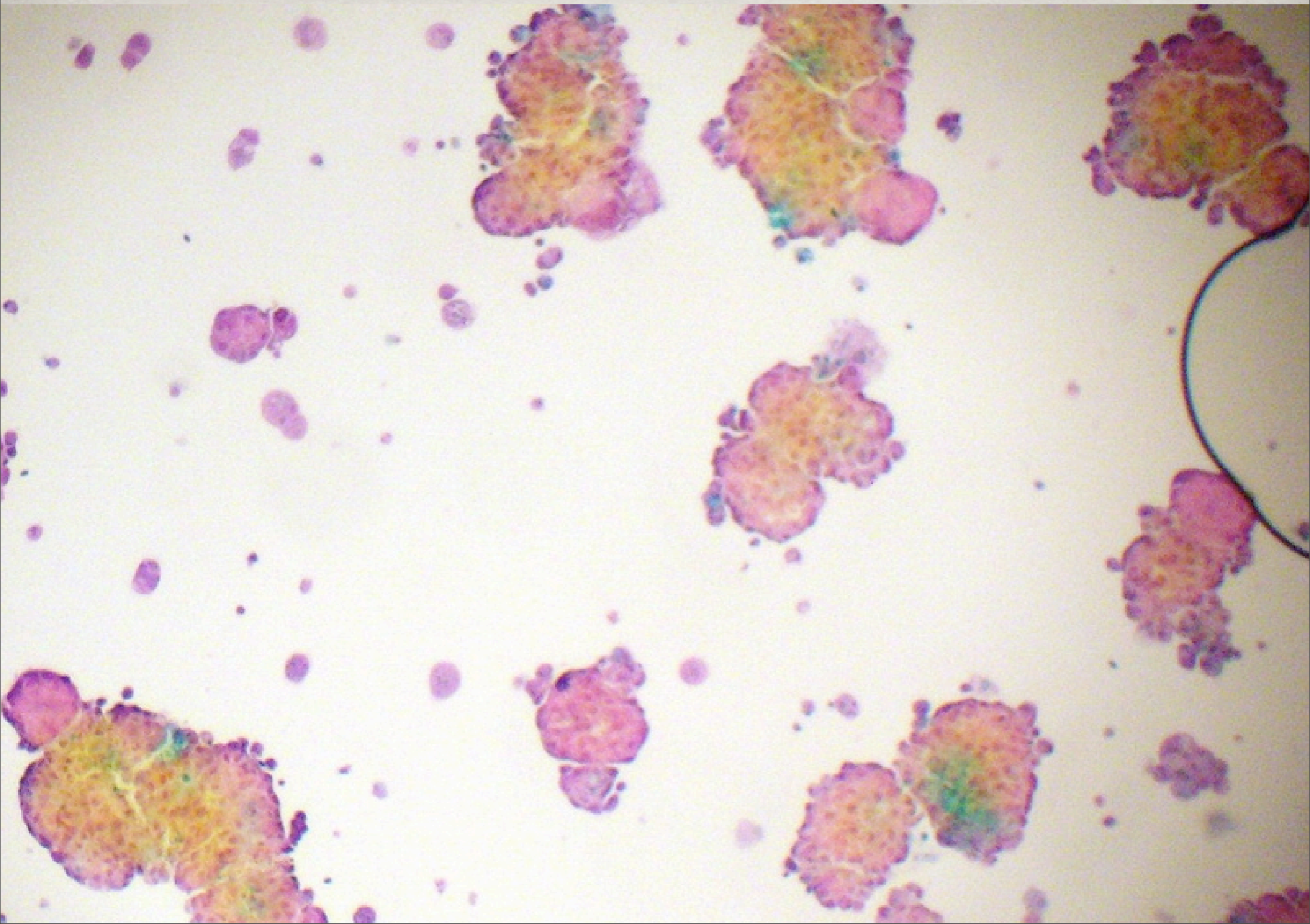


Sunitinib



Sorafenib

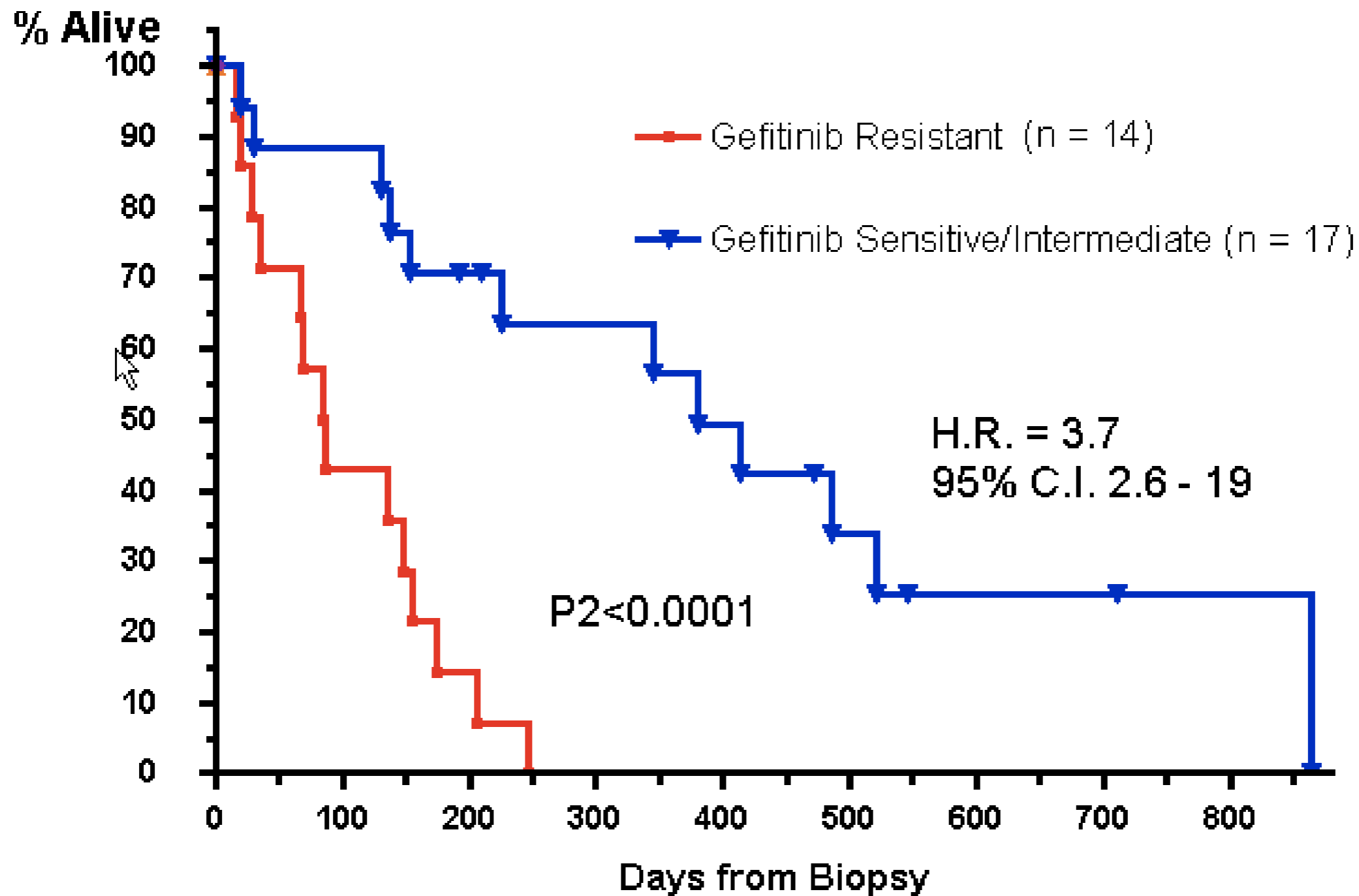




Presence of a molecular target is not sufficient

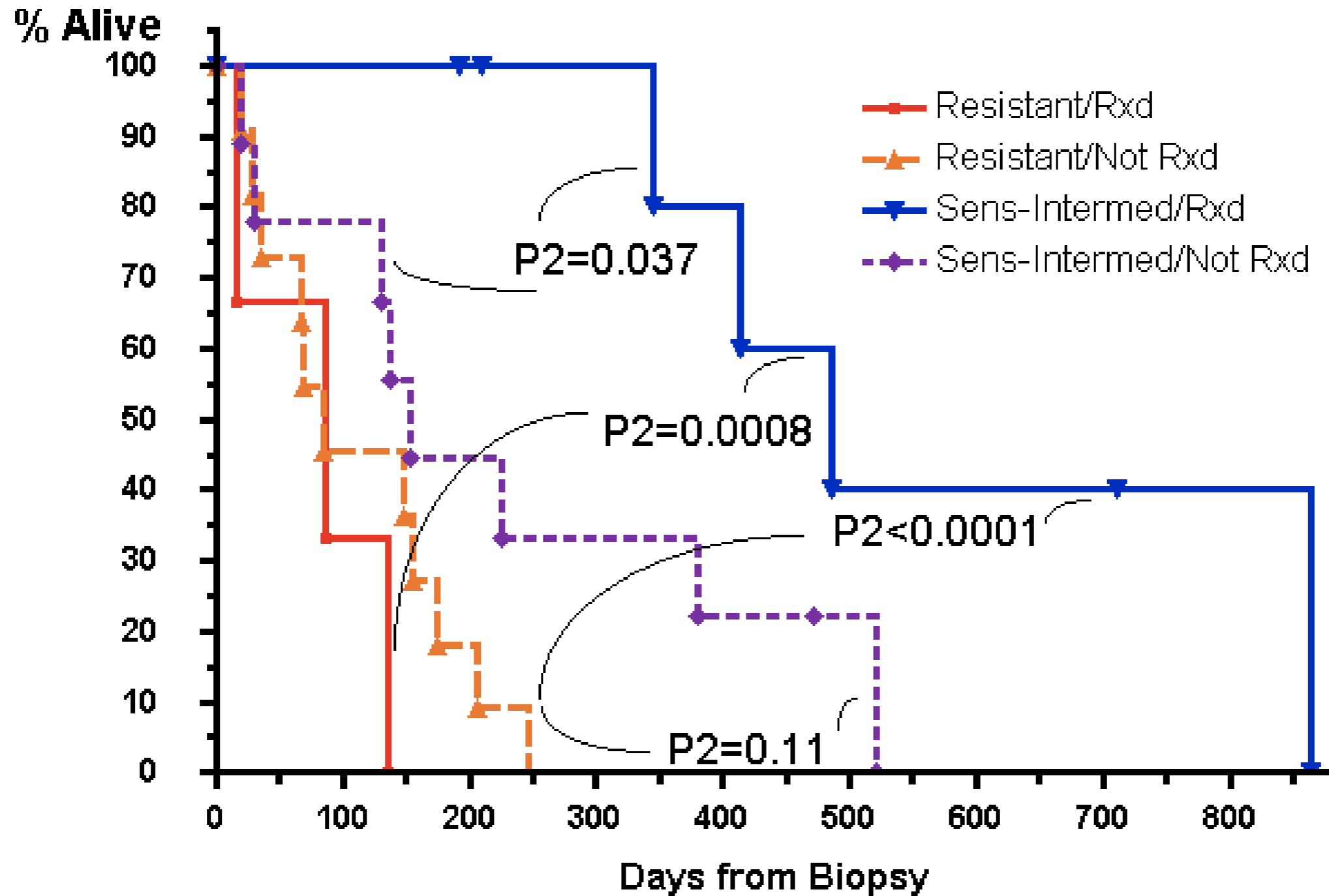
- if the drug doesn't get in
- if the drug is pumped out
- if the drug is metabolized

Previously-Treated NSCLC, Survival as Function of Cell Death Assay Results (as Reported Prospectively)



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Previously-Treated NSCLC, Survival as Function of Assay Results (as Reported Prospectively) and Gefitinib/Erlotinib Treatment



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Disease-specific activity of gefitinib

Gefitinib Database

Query Summary Statistics Gefitinib (Iressa)

	DISC		MIT		ATP		Average	
	HI	Lo	Hi	Lo	Hi	Lo	Hi	Lo
Freq.	699	697	596	596	0	0	566	565
Mean	53	76	63	86	0	0	59	82
Std.Dev	30	24	29	22	0	0	28	21
Extr. Res.	> 83	100	91	108	0	0	87	103
Resistant	> 68	88	77	97	0	0	73	92
Sensitive	< 38	64	48	75	0	0	45	72

CELL SURVIVAL INFORMATION

Viability

Spec.Count
Mean
Std.Dev.

713
0.74
0.33

MTTD

637
0.55
0.26

CELL CLUSTERING INFORMATION

% Day0 % Day4

704 692
65.63 79.23
24.75 17.85

Size0 Size4

704 692
7.21 17.52
5.95 19.90

CELL CLUSTER DENSITY COUNT

Zero Loose Medium Tight

Day0 55 107 527 15
Day4 30 89 482 91

[Alt][D]=Drugs

[Alt][C]=Convert to DBF

[Alt][P]=Print

Gefitinib

Untreated + Treated Patients

<-- More sensitive

More resistant -->

Carcinoid

Renal Cell

NSCLC

Colon

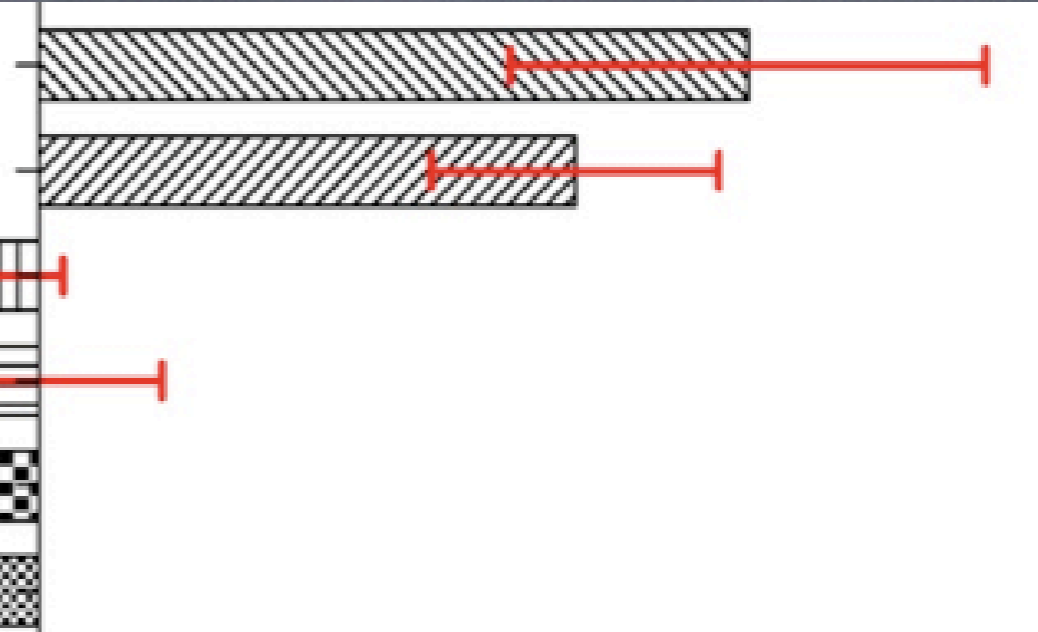
Ovarian

Breast

35 45 55 65 75 85 95

Mean % Control Cell Survival

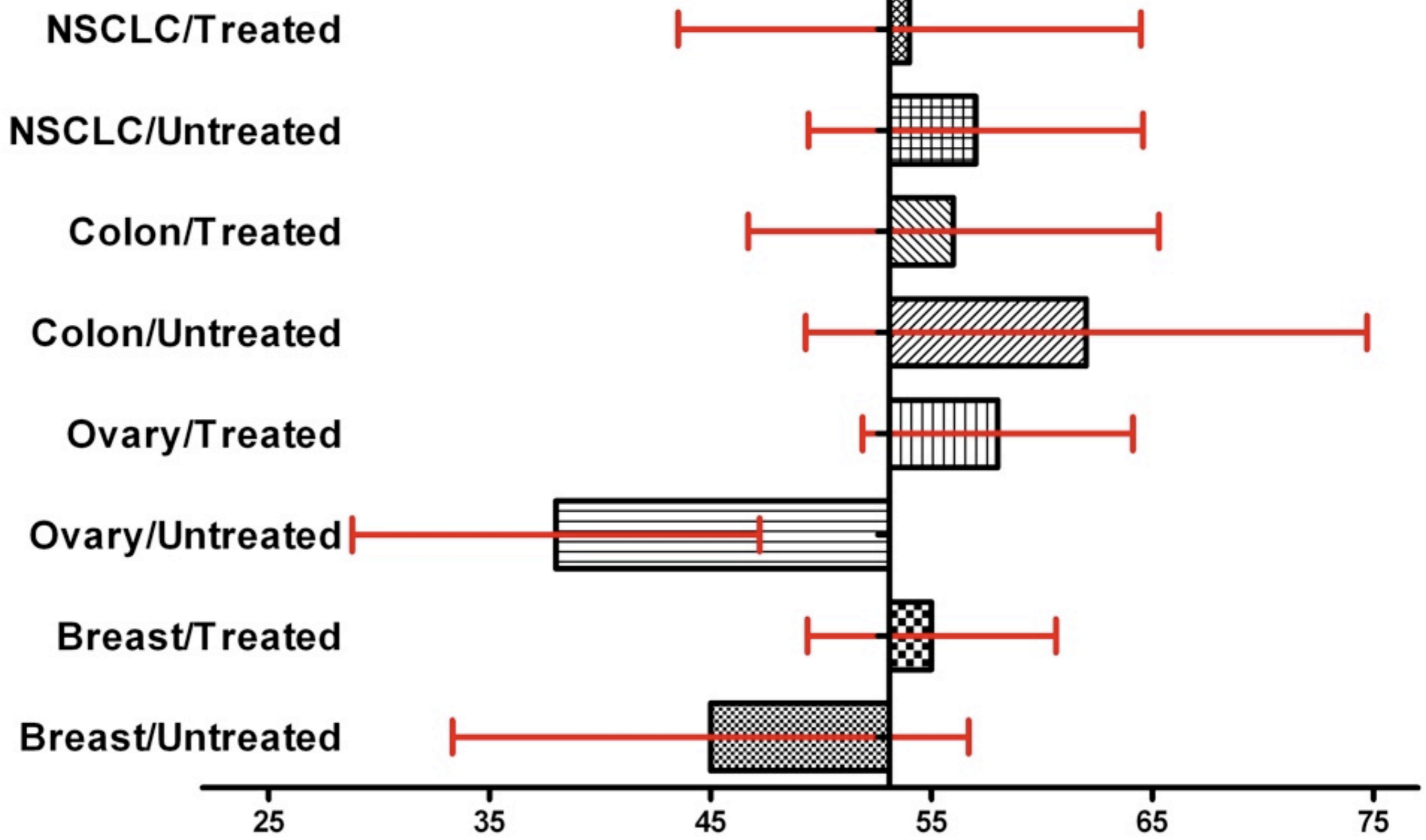
Red Bars 95% Confidence Intervals



Gefitinib

<-- More sensitive

More resistant -->



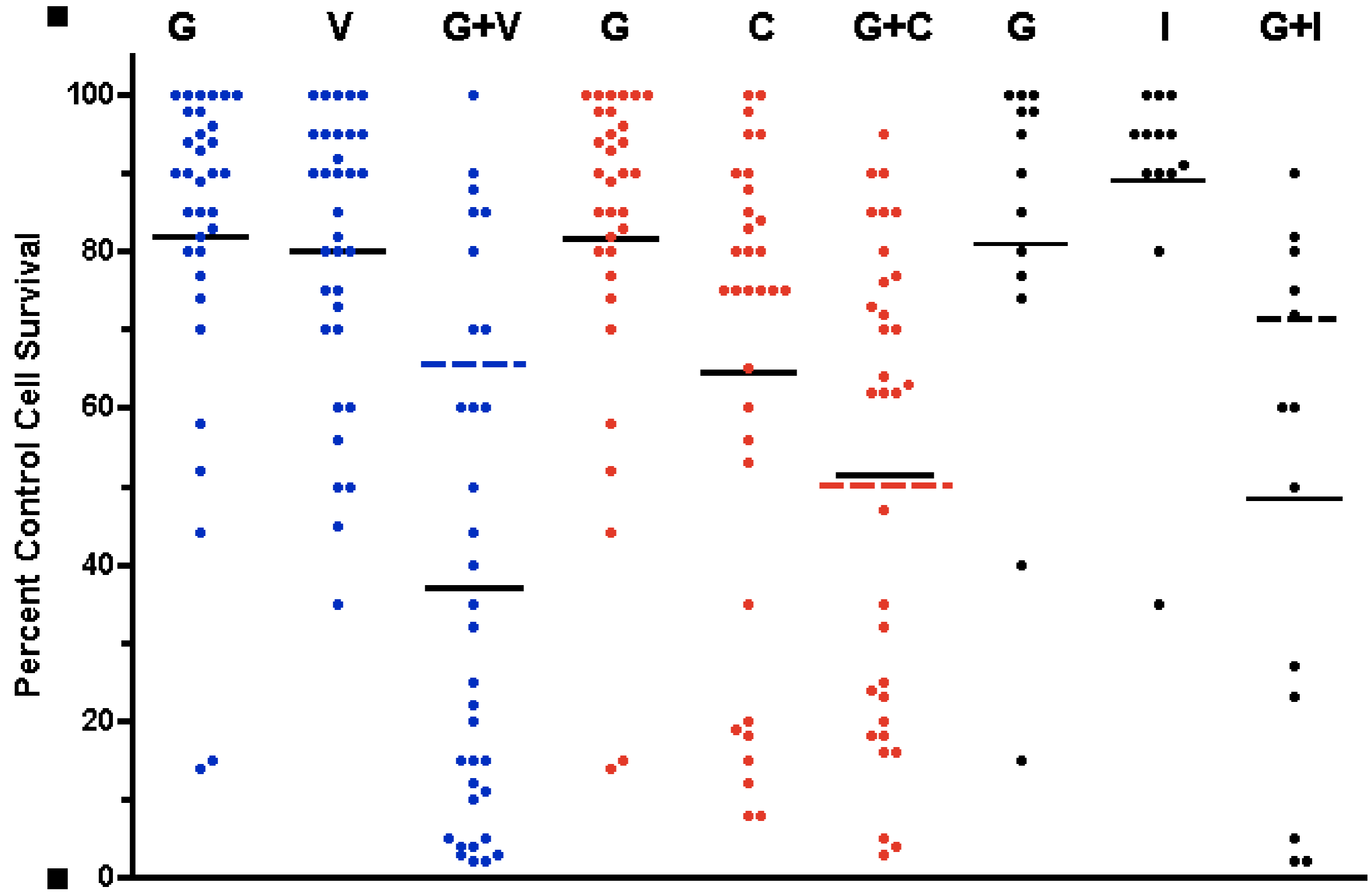
Mean % Control Cell Survival

Red Bars 95% Confidence Intervals

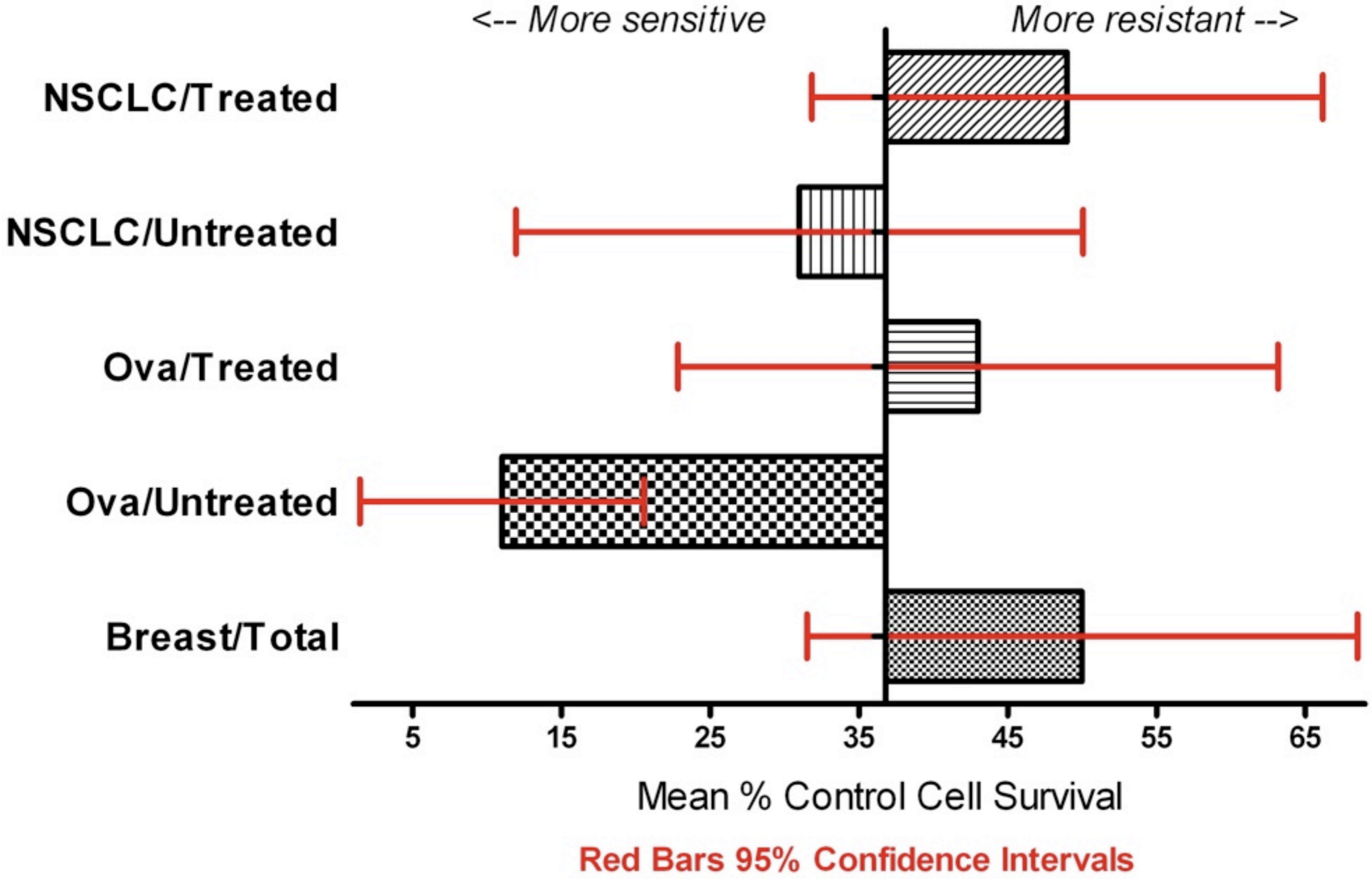
Colored dashed lines = expected mean result if additive; Solid black lines = actual mean.

Gefitinib is Synergistic with Vinorelbine, but Only Additive with Cisplatin

G = gefitinib; V = vinorelbine;
C = cisplatin; I = irinotecan



Gefitinib + Vinorelbine



Microvascular viability assay for bevacizumab and related agents.

- Lack of time
- Patent pending
- Send me an e-mail and I'll send you PowerPoint slides detailing method and preliminary results before Jan 1, 2007.
- mail@weisenthal.org (Larry Weisenthal)

Conclusions: fresh human tumor cell culture assays with cell death endpoints

- Predict for both response and survival
- Identify disease-specific drug activity
- Self generate “gold standards” in the case of new drugs
- Identify synergistic drug combinations
- May be rationally utilized to improve drug selection in patient treatment and to improve patient selection in clinical trials.