Individualized pharmacotherapy with Cell Culture Assays

Larry Weisenthal http://weisenthal.org

Presentation outline

- Need for individualized therapy (efficacy,cost)
- Appropriate criteria for evaluating predictive tests: Accuracy vs. "Efficacy," example: Estrogen Receptor IHC, Oncotype Dx multigene expression test
- Very brief and broad overview of data pertaining to cell culture assays
- Detailed consideration of a single example: chronic lymphocytic leukemia
- Cell culture assays for "targeted" drugs
- Cell culture assay for anti-microvascular drugs

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Classic Chemotherapy Research Paradigm

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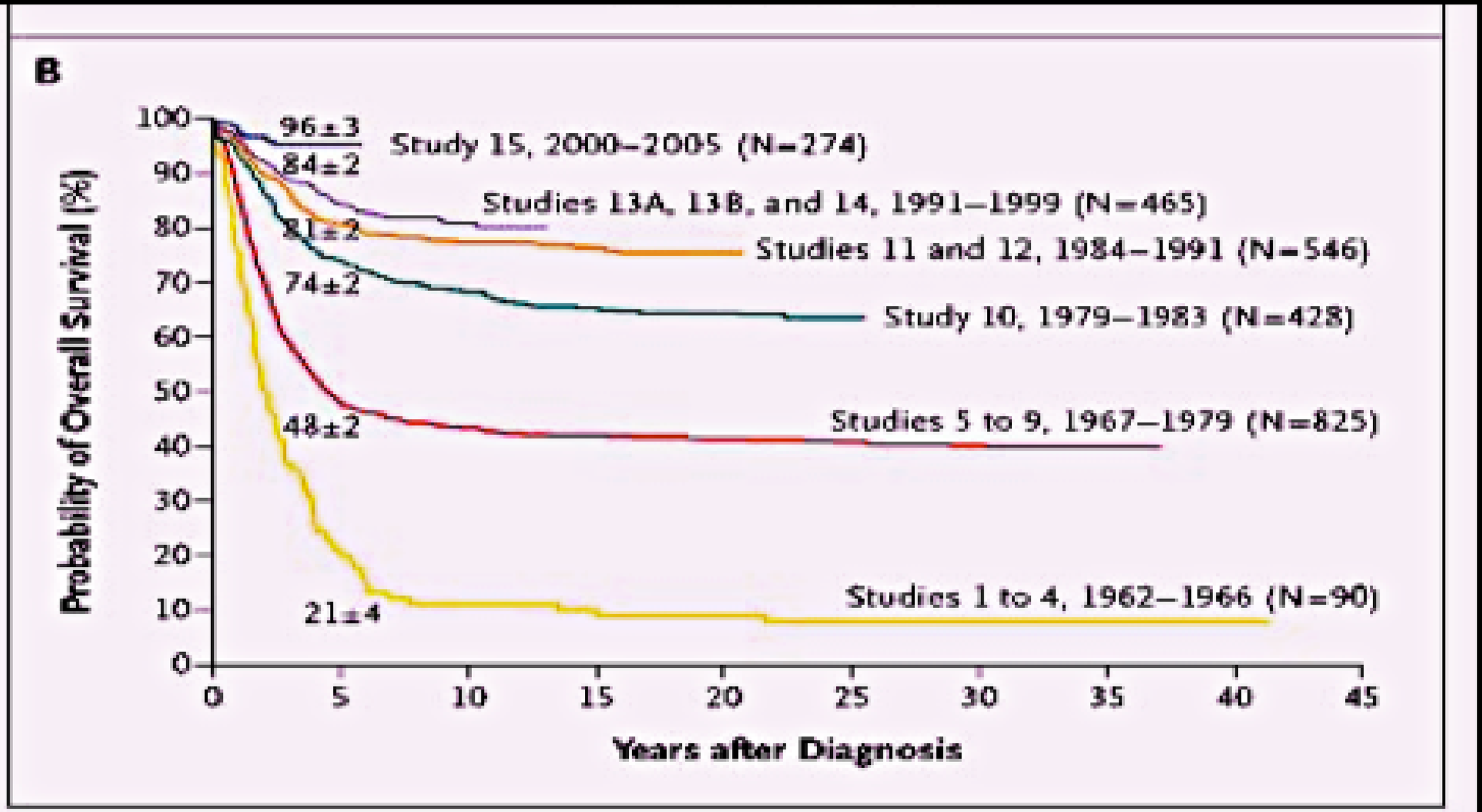
It worked the first time it was tried, in 1950, but it has virtually never again worked.

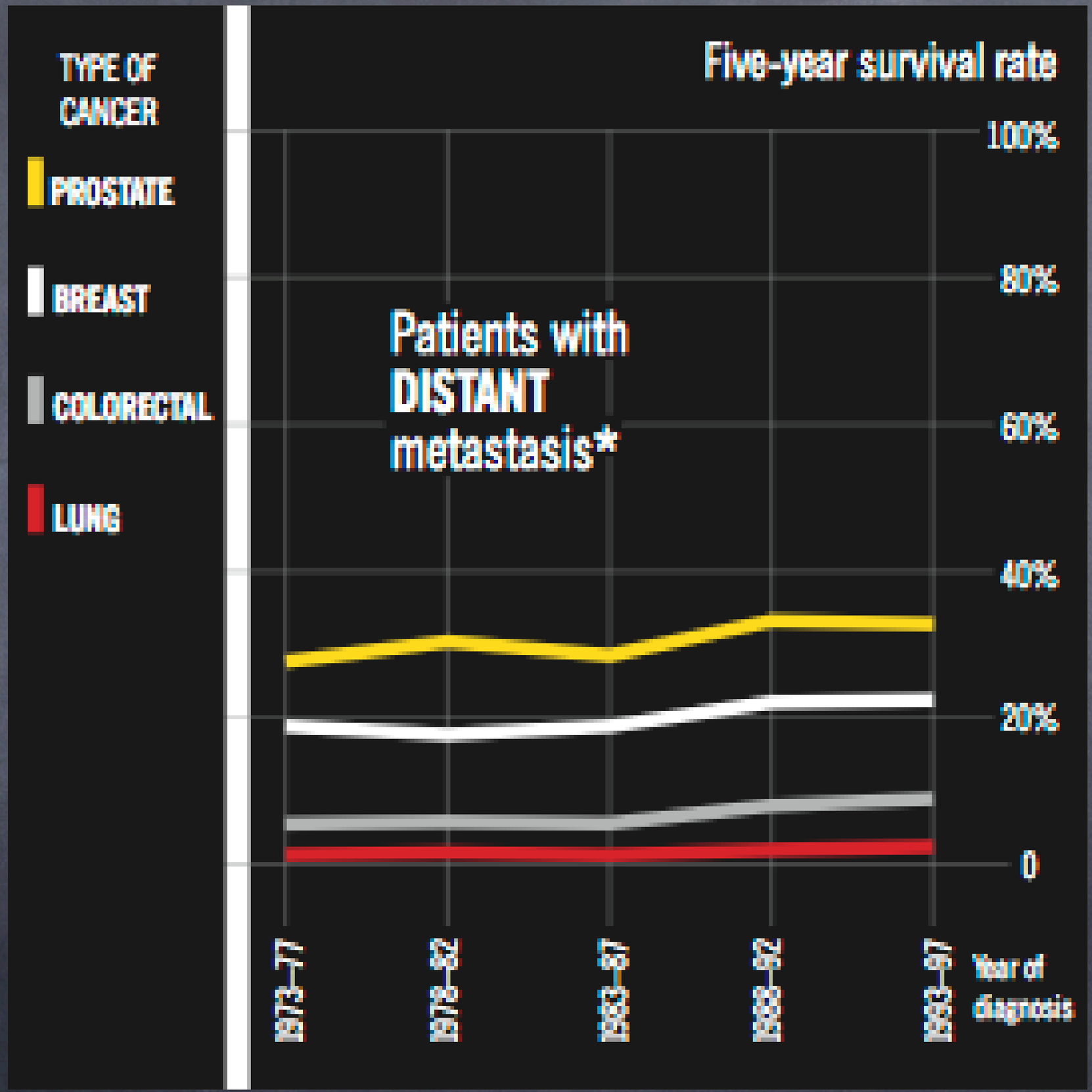
Any experiment which has failed 1,000 consecutive times should be viewed with suspicion. - Martin Apple, U of Cal San Francisco, 1969

Overall Survival in 2628 Children with Newly Diagnosed ALL



Pui C and Evans W. N Engl J Med 2006;354:166-178

The NEW ENGLAND JOURNAL of MEDICINE


TYPE OF CANCER
PROSTATE
BREAST
COLORECTAL
LUNG
Five-year survival rate
100%
80%
60%
40%
20%
0
Patients with DISTANT metastasis*
2007–13
2008–14
2009–15
2010–16
2011–17
Year of diagnosis

Official NCI statement on “state of the art” chemotherapy for metastatic breast cancer

- **Anthracyclines.**
  - **Doxorubicin.**
  - **Epirubicin.**
  - **Liposomal doxorubicin**
  - **Mitoxantrone.**
- **Taxanes.**
  - **Paclitaxel.**
  - **Docetaxel.**
  - **Albumin-bound nanoparticle paclitaxel (ABI-007 or Abraxane).**
- **Alkylating agents.**
  - **Cyclophosphamide.**
- **Fluoropyrimidines.**
  - **Capecitabine.**
  - **5-FU.**
- **Antimetabolites.**
  - **Methotrexate.**
- **Vinca alkaloids.**
  - **Vinorelbine.**
  - **Vinblastine.**
  - **Vincristine.**
- **Platinum.**
  - **Carboplatin.**
  - **Cisplatin.**
- **Other.**
  - **Gemcitabine.**

Drug Combinations.

- **CA: cyclophosphamide and doxorubicin.**
- **Docetaxel and doxorubicin.**
- **CAF: cyclophosphamide, doxorubicin, 5-fluorouracil.**
- **CMF: cyclophosphamide, methotrexate, 5-fluorouracil.**
- **Doxorubicin and paclitaxel.**
- **Docetaxel and capecitabine.**
- **Vinorelbine and epirubicin.**

“Whether single-agent chemotherapy or combination chemotherapy is preferable for first-line treatment is unclear.”

“At this time, no data support the superiority of any particular regimen.”

NCI PDQ Recommended Equally-Acceptable Treatments
http://www.cancer.gov/cancertopics/pdq/treatment/breast/HealthProfessional/page8

Official NCI statement on “state of the art” chemotherapy for Non Hodgkin’s Lymphoma

Rituximab and combinations:

- **Rituximab alone.**
- **R-F: rituximab + fludarabine.**
- **R-CVP: rituximab + cyclophosphamide + vincristine + prednisone.**
- **R-CHOP: rituximab + cyclophosphamide + doxorubicin + vincristine + prednisone.**
- **R-FM: rituximab + fludarabine + mitoxantrone.**
- **R-FCM: rituximab + fludarabine + cyclophosphamide + mitoxantrone.**

Purine nucleoside analog:

- **Fludarabine.**
- **2-chlorodeoxyadenosine**.

Oral alkylating agents (with or without steroids):

- **Cyclophosphamide**
- **Chlorambucil.**

Combination chemotherapy alone:

- **CVP: cyclophosphamide + vincristine + prednisone.**
- **C-MOPP: cyclophosphamide + vincristine + procarbazine + prednisone.**
- **CHOP: cyclophosphamide + doxorubicin + vincristine + prednisone.**
- **FND: fludarabine + mitoxantrone ± dexamethasone.**

“Currently, no randomized trials guide clinicians about the initial choice of rituximab, nucleoside analogs, alkylating agents, combination chemotherapy, radiolabeled monoclonal antibodies, or combinations of these options.”

“Although the addition of rituximab to chemotherapy reproducibly improves response rates and failure-free survival in randomized clinical trials, as yet, no improvement in overall survival has been observed.”

NCI PDQ: http://www.cancer.gov/cancertopics/pdq/treatment/adult-non-hodgkins/HealthProfessional/page7

Stages III & IV Ovarian Cancer - Paclitaxel/Platinum Combinations Versus Comparator Arms in Trials

Trial	Treatment Regimens	N	PFS (mo)	OS (mo)
GOG-132	**Paclitaxel** (135 mg/m^2, 24 h) and **cisplatin** (75 mg/m^2)	201	14.2	**26.6**
	Cisplatin (100 mg/m^2)	200	16.4	**30.2**
	Paclitaxel (200 mg/m^2, 24 h)	213	11.2*	**26.0**
MRC-ICON	**Paclitaxel** (175 mg/m^2, 3 h) and **carboplatin** AUC 6	478	17.3	**36.1**
	Carboplatin AUC 6	943	16.1	**35.4**
	Paclitaxel (175 mg/m^2, 3 h) and **carboplatin** AUC 6	232	17.0	**40.0**
	Cyclophosphamide (750 mg/m^2) and **doxorubicin** (75 mg/m^2) and **cisplatin** (75 mg/m^2)	421	17.0	**40.0**
GOG-111	**Paclitaxel** (135 mg/m^2, 24 h) and **cisplatin** (75 mg/m^2)	184	18.0	**38.0**
	Cyclophosphamide (750 mg/m^2) and **cisplatin** (75 mg/m^2)	202	13.0*	24.0*
OV-10	**Paclitaxel** (175 mg/m^2, 3 h) and **cisplatin** (75 mg/m^2)	162	15.5	**35.6**
	Cyclophosphamide (750 mg/m^2) and **cisplatin** (75 mg/m^2)	161	11.5*	25.8*

NCI PDQ http://www.cancer.gov/cancertopics/pdq/treatment/ovarianepithelial/HealthProfessional/ Table1

*** = statistically inferior result p < .001 - .05**

Official NCI statement on “state of the art” chemotherapy for advanced lung (NSC) cancer

Regimens associated with similar survival outcomes

- Cisplatin plus vinblastine plus mitomycin]
- Cisplatin plus vinorelbine
- Cisplatin plus paclitaxel
- Cisplatin plus docetaxel
- Cisplatin plus gemcitabine
- Carboplatin plus paclitaxel

“The results support further evaluation of chemotherapeutic approaches for both metastatic and locally advanced NSCLC”

“…the efficacy of current platinum-based chemotherapy combinations is such that no specific regimen can be regarded as standard therapy.”

“Appropriate patients are candidates for clinical trials that evaluate the role of platinum-based and nonplatinum-based chemotherapy.”

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Coming soon: Bevacizumab + Erlotinib $13,500 per month

Sunitinib, Sorafenib: Each $10,000 per month

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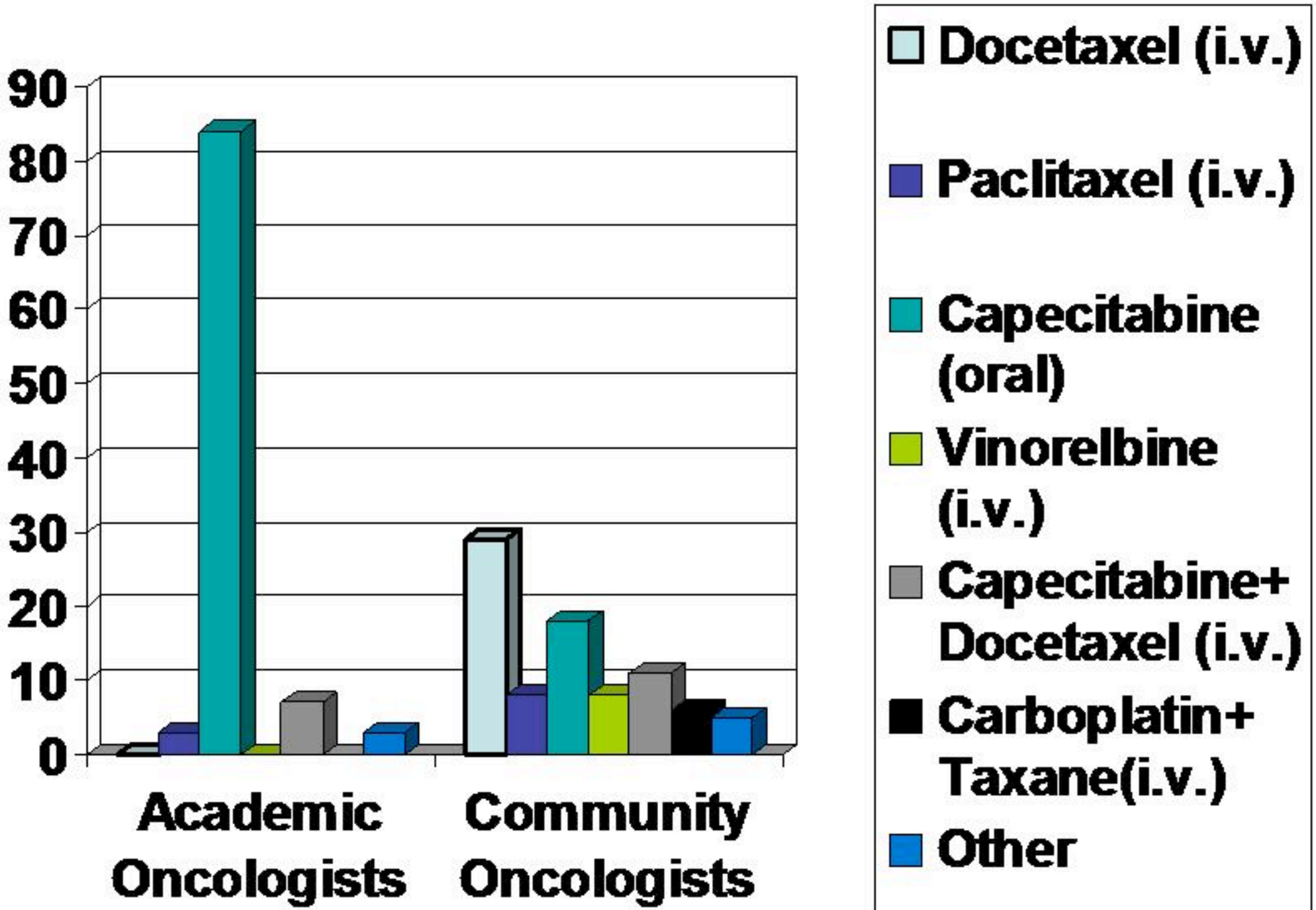
Treatment of Metastatic Breast Cancer by Academic Oncologists and Community Oncologists



Love,N. http://PatternsOfCare.com

Individualized Chemotherapy Assay Development

Year	Cell Death Assays	Proliferation Assays	Static Gene or Protein
1930			
	Trypan Blue (‘36)		
1950	**Tetrazolium/SDI (‘54)**		
1960			
1970			
		Clonogenic (‘76)	**Steroid Receptor (’75) (radioligand binding)**
1980	**DiSC (‘81)** **FCP (’81)**	- 3H-TdR	
	- EVA **ChemoFx**	- Capillary	
	- TRAK		
	ATP (’83)		**IHC Various (’80’s – ’90’s)**
1990	**MTT (’86)**		ER,PR,Her2/Neu
	- HDRA FMCA (’90)		
2000			
	Putative “specific” apoptotic: MICK,Annexin, Tunel, Caspase		**Molecular Various Single gene** TS, EGFR,Her2n
2007			**Multigene**
	Microvascular Viability (MVVA) (’06)		e.g. Oncotype Dx

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Accuracy vs "Efficacy"

There have been no prospective clinical trials that have demonstrated that there is an improved survival among patients in whom chemosensitivity assays were used to positively select chemotherapy regimens. Therefore, chemosensitivity assays are considered to be investigational, and are not a service covered by this medical plan. - Aetna Medical Policy, 2006

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."

Breast Cancer
Vol. 13 No. 1 January 2006

Original Article

Immunohistochemical Evaluation of Hormone Receptor Status for Predicting Response to Endocrine Therapy in Metastatic Breast Cancer

Hiroko Yamashita*[1], Yoshiaki Ando*[1], Mariko Nishio*[1], Zhenhuan Zhang*[1], Maho Hamaguchi*[1], Keiko Mita*[1], Shunzo Kobayashi*[1], Yoshitaka Fujii*[1], and Hirotaka Iwase*[2]

*[1]*Oncology and Immunology, Nagoya City University Graduate School of Medical Sciences,* *[2]*Breast and Endocrine Surgery, Kumamoto University, Japan.*

IHC Estrogen Receptor Study

56 Patients, Retrospective

Response rate for ER Positive = 56%

Response rate for ER negative = 20%

P2 = 0.03

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer

Soonmyung Paik, M.D., Steven Shak, M.D., Gong Tang, Ph.D.,
Chungyeul Kim, M.D., Joffre Baker, Ph.D., Maureen Cronin, Ph.D.,
Frederick L. Baehner, M.D., Michael G. Walker, Ph.D., Drew Watson, Ph.D.,
Taesung Park, Ph.D., William Hiller, H.T., Edwin R. Fisher, M.D.,
D. Lawrence Wickerham, M.D., John Bryant, Ph.D.,
and Norman Wolmark, M.D.

Oncotype Dx

Retrospective correlations between outcomes of patients treated a decade ago and present day tests performed on archival paraffin blocks.

More than half of all US oncologists now using this ($3,600) test. Medicare and major insurance companies pay for the test.

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The issue of non-"real world" test conditions

Oncotype Dx NEJM Methods

No samples from trial B-14 were used for prior testing or training. The prospectively defined assay methods and end points were finalized in a protocol signed on August 27, 2003. RT-PCR analysis was initiated on September 5, 2003, and RT-PCR data were transferred to the NSABP for analysis on September 29, 2003.

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These are NOT "real world" test conditions!

...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
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Experience Tomorrow's Cancer Testing Laboratory Today.

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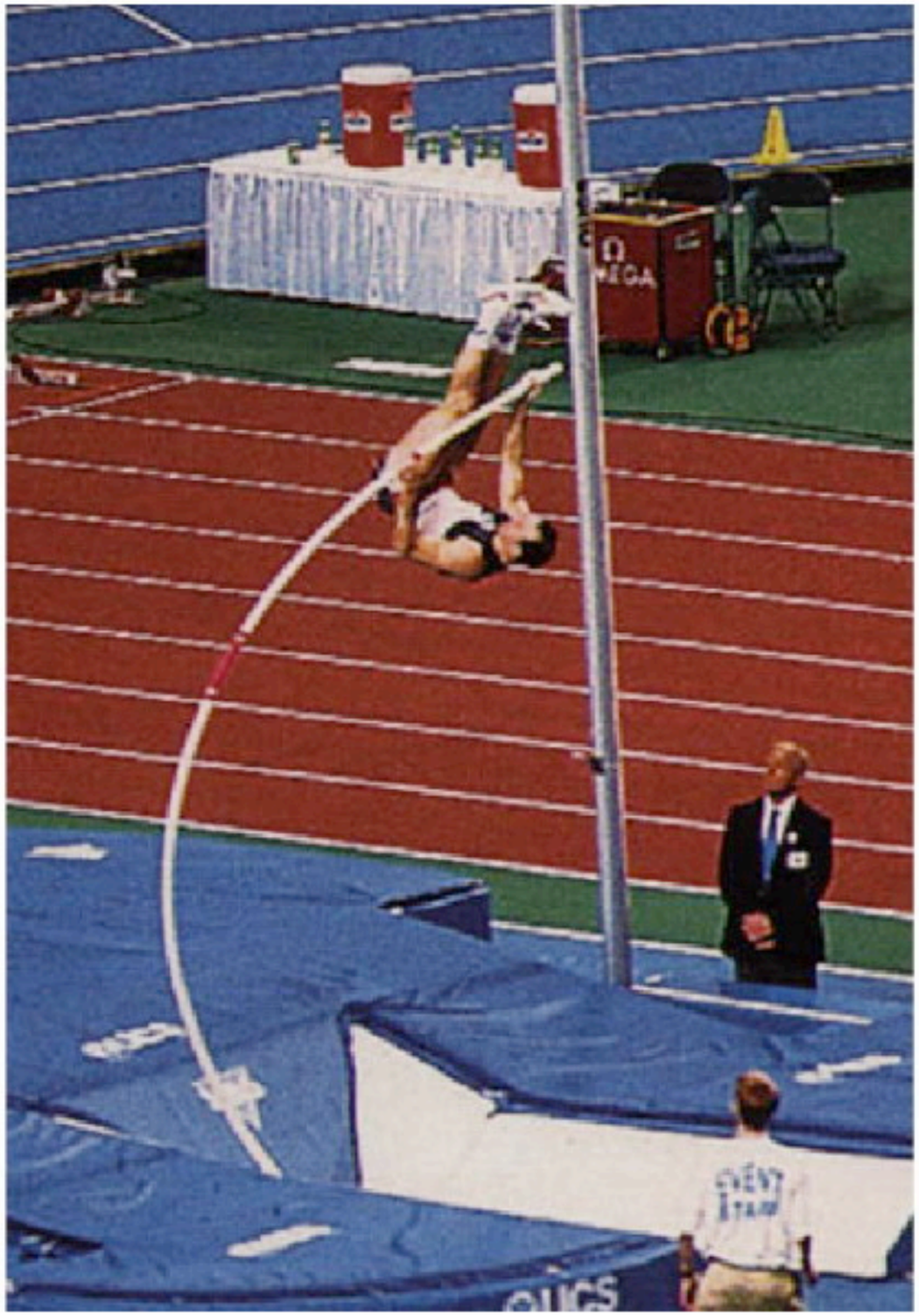
The "Bar" for Predictive Tests



Standard for

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

Standard for Genomic Tests

Standard for

Cell culture assays

Standard for Cell Culture Tests

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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.
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http://weisenthal.org (Click on "Tokyo Meeting PDF")

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

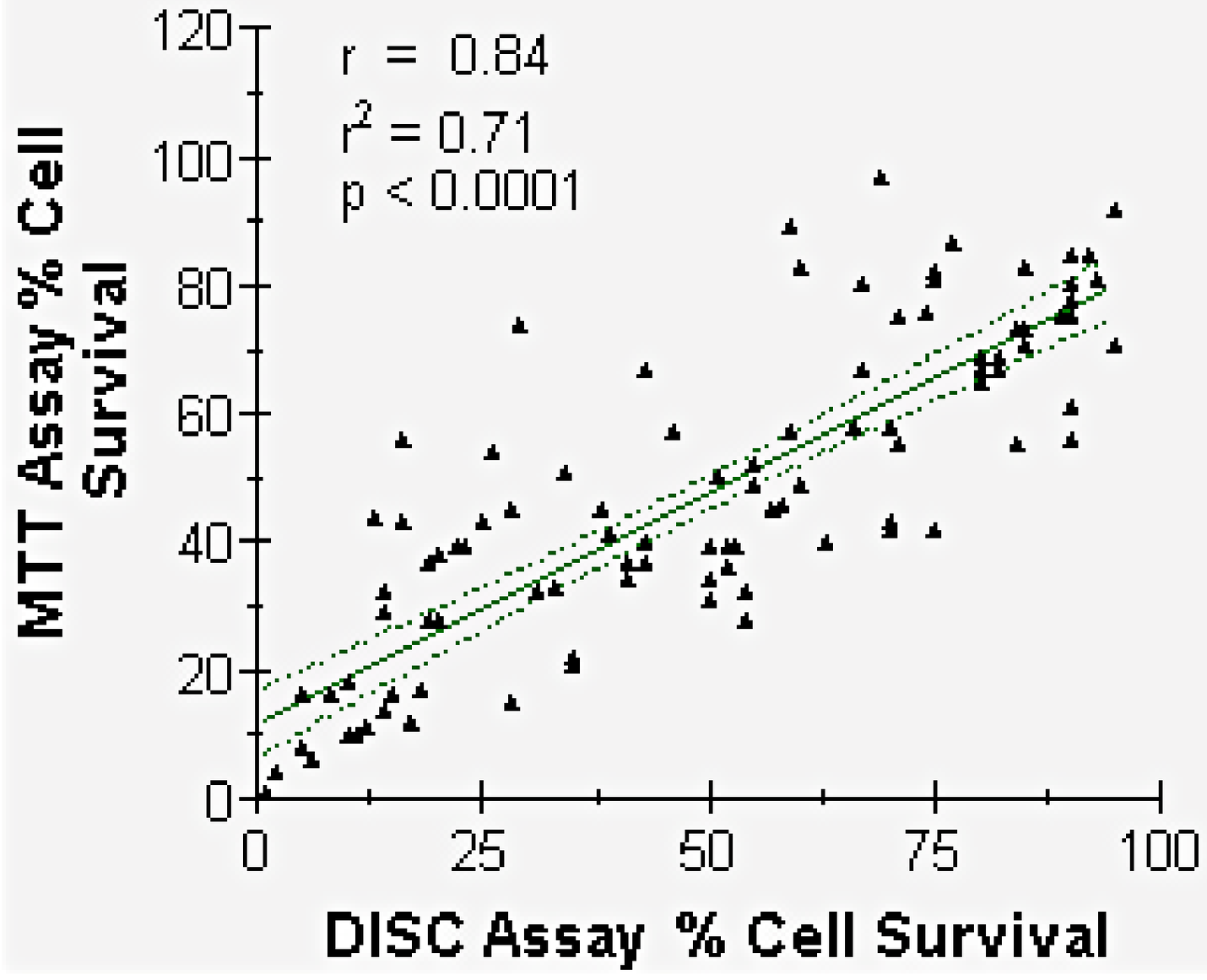
Cell death

- 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



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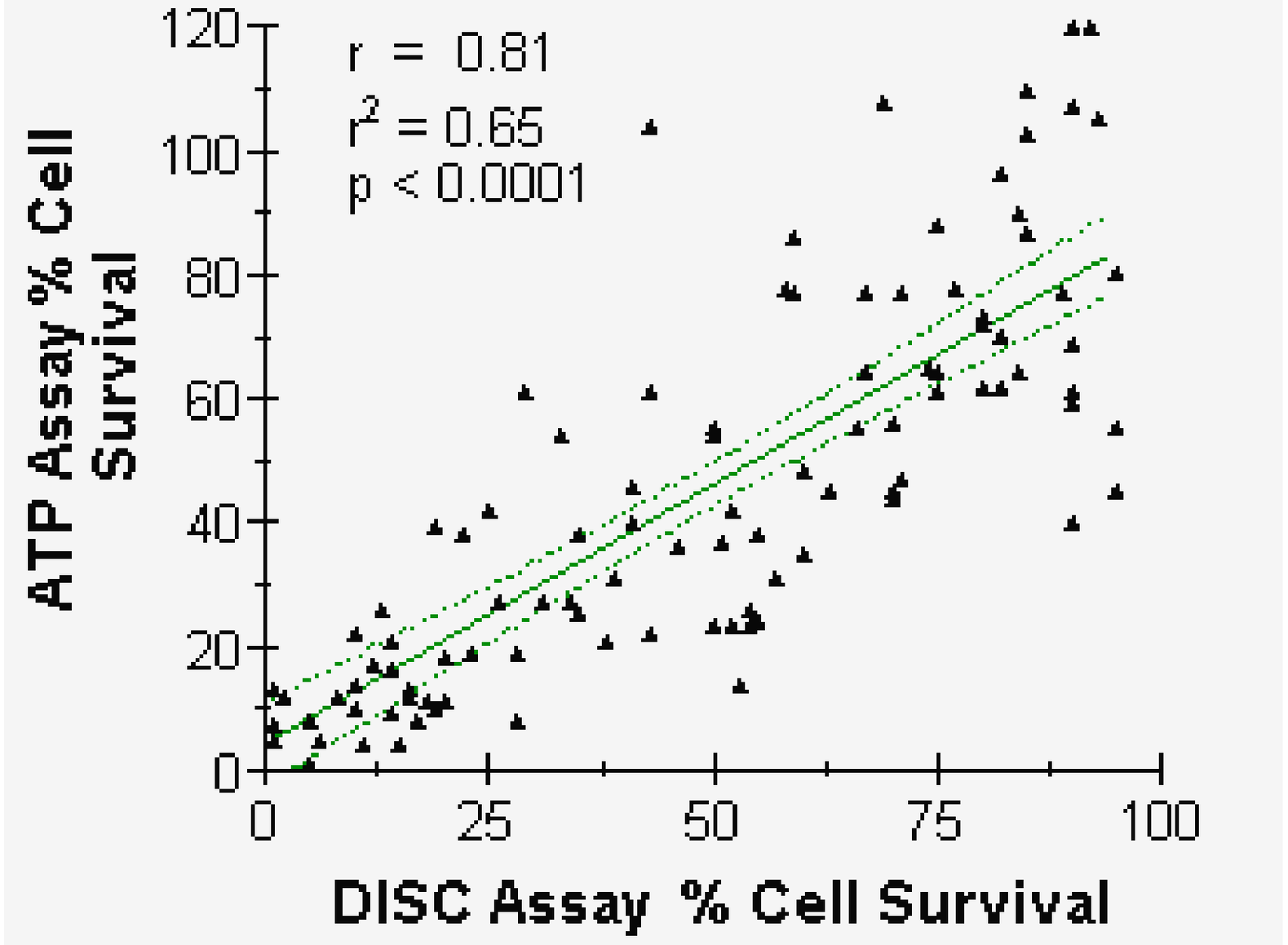

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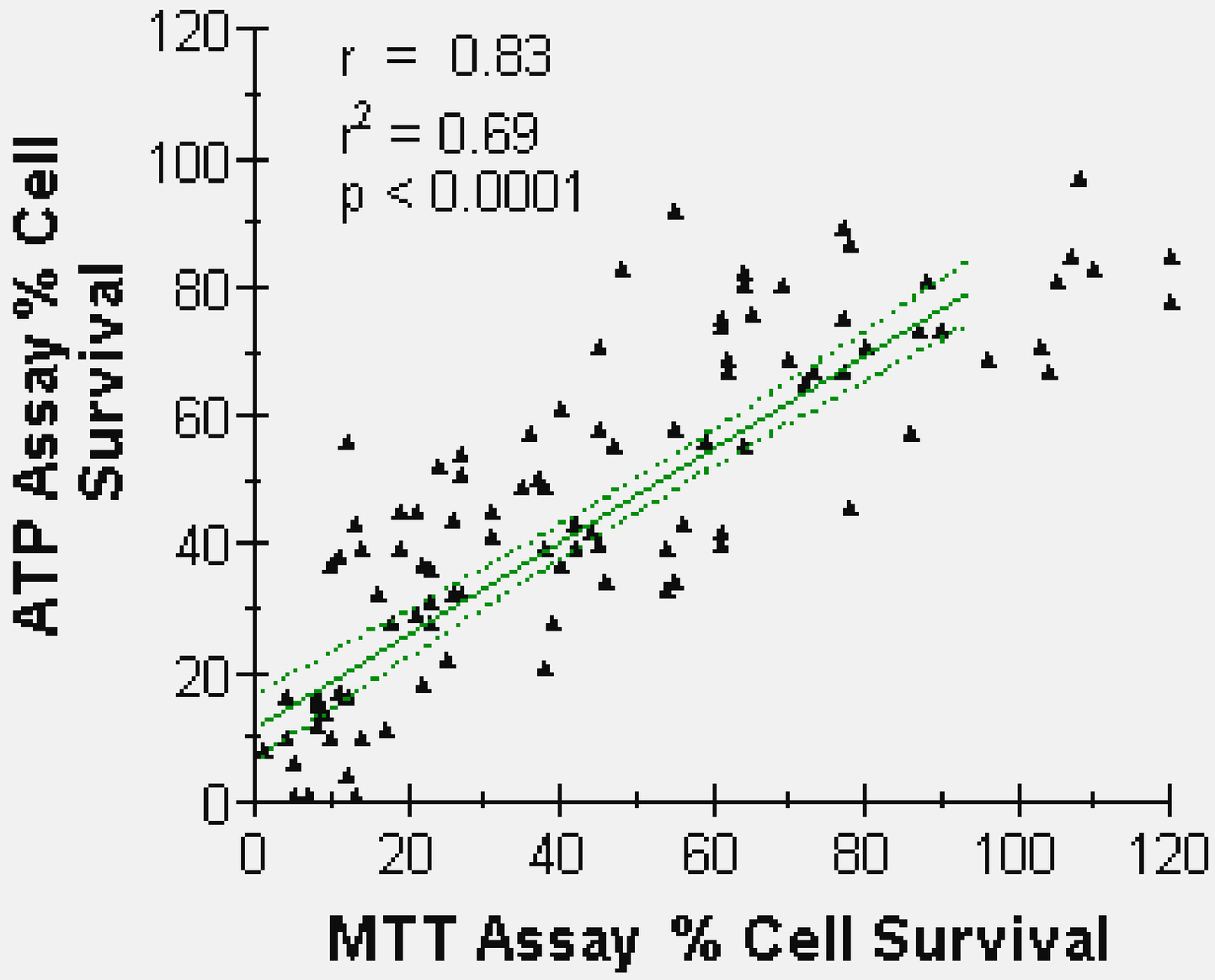
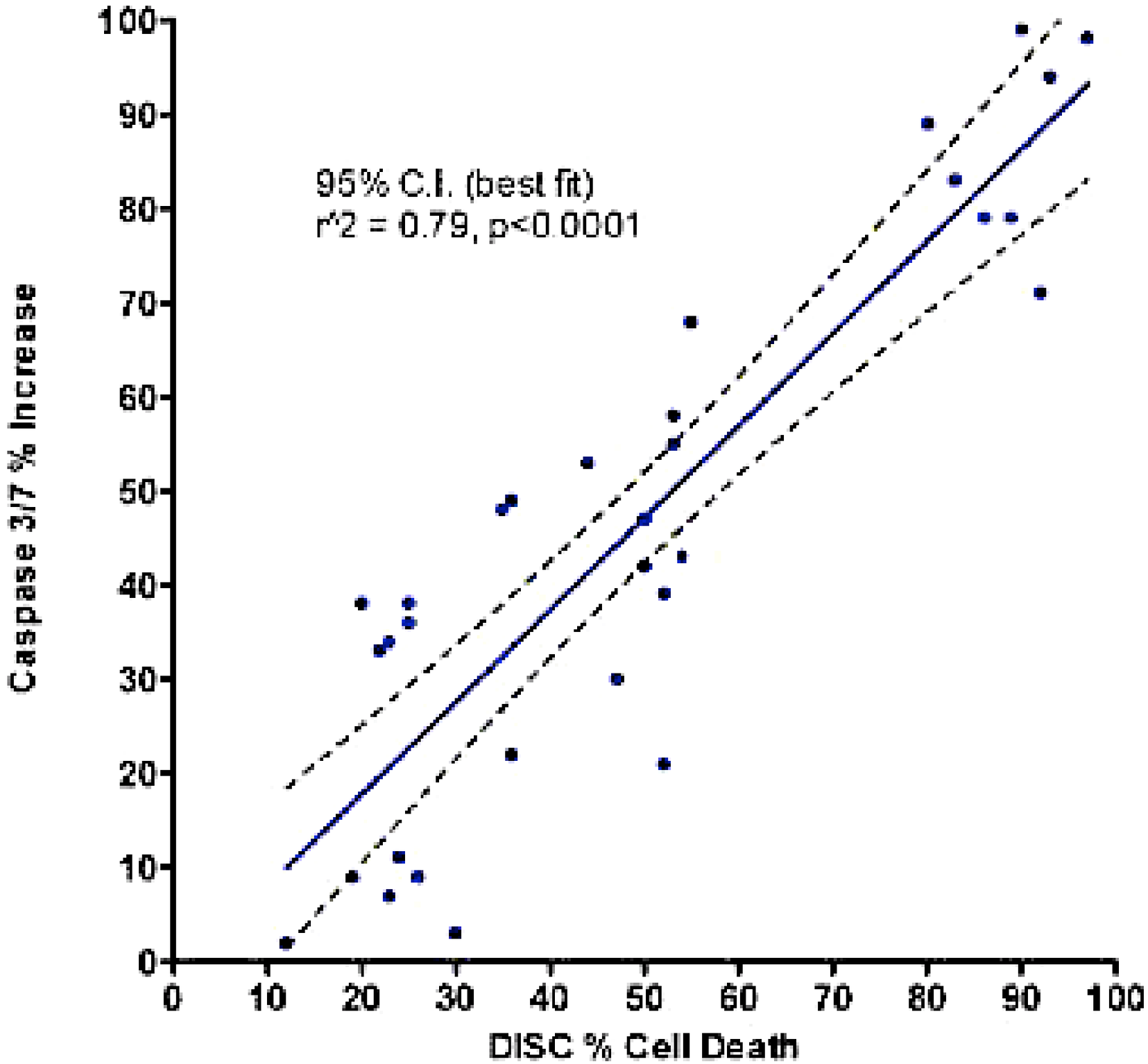
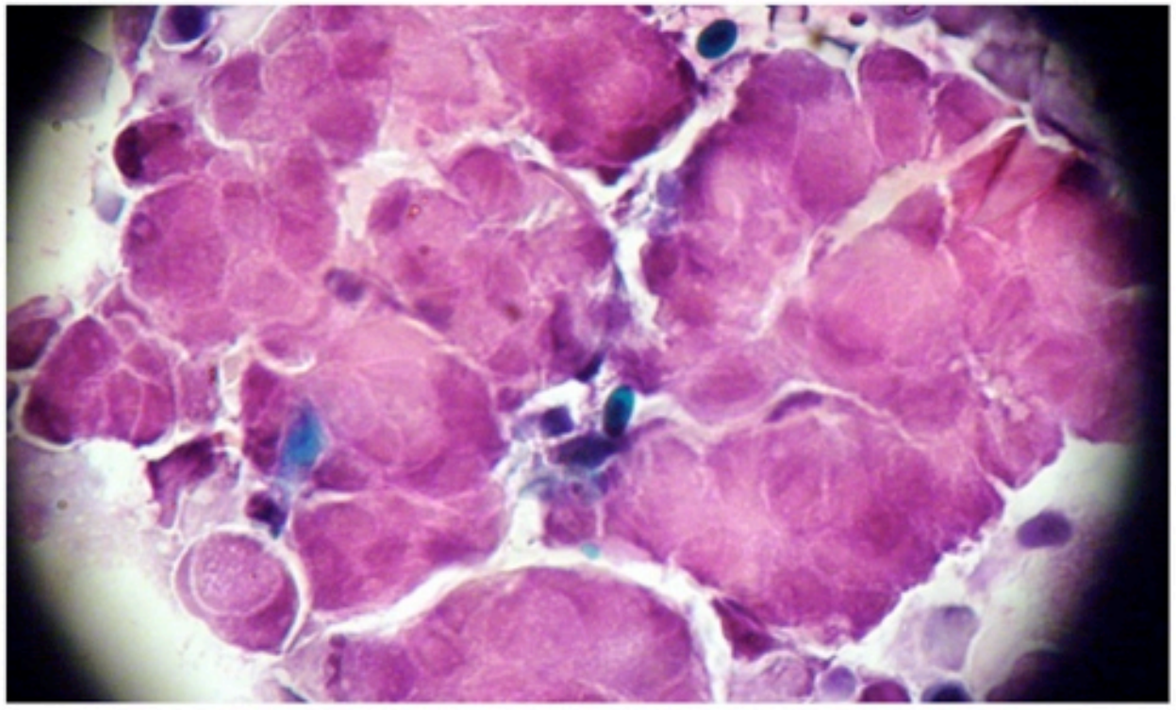


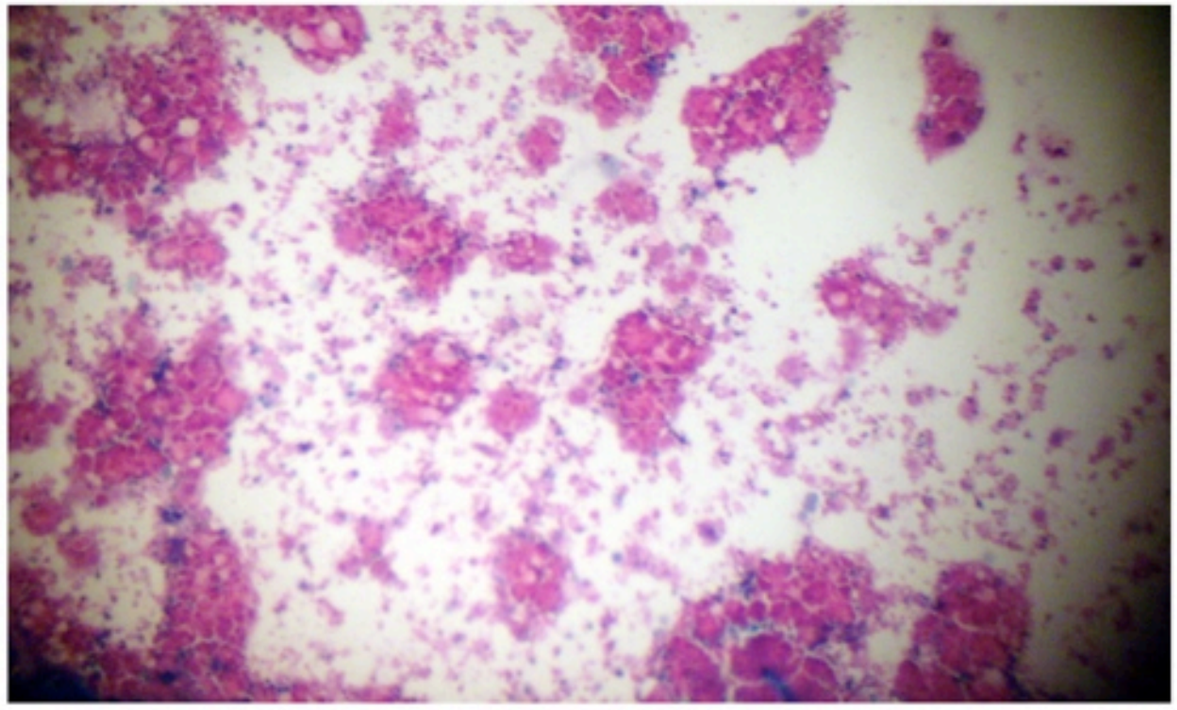
96 hr. DISC Assay vs 42 hr Caspase 3/7 expression

Ovarian Cancer, 16 drugs; 2 concentrations

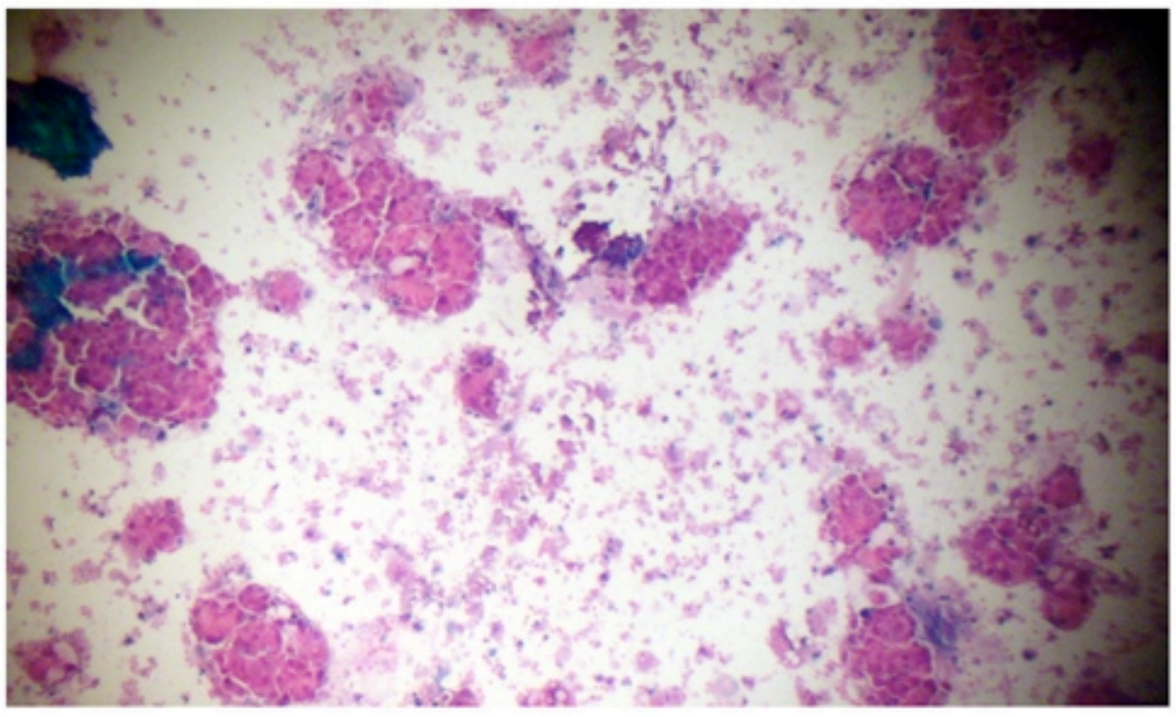




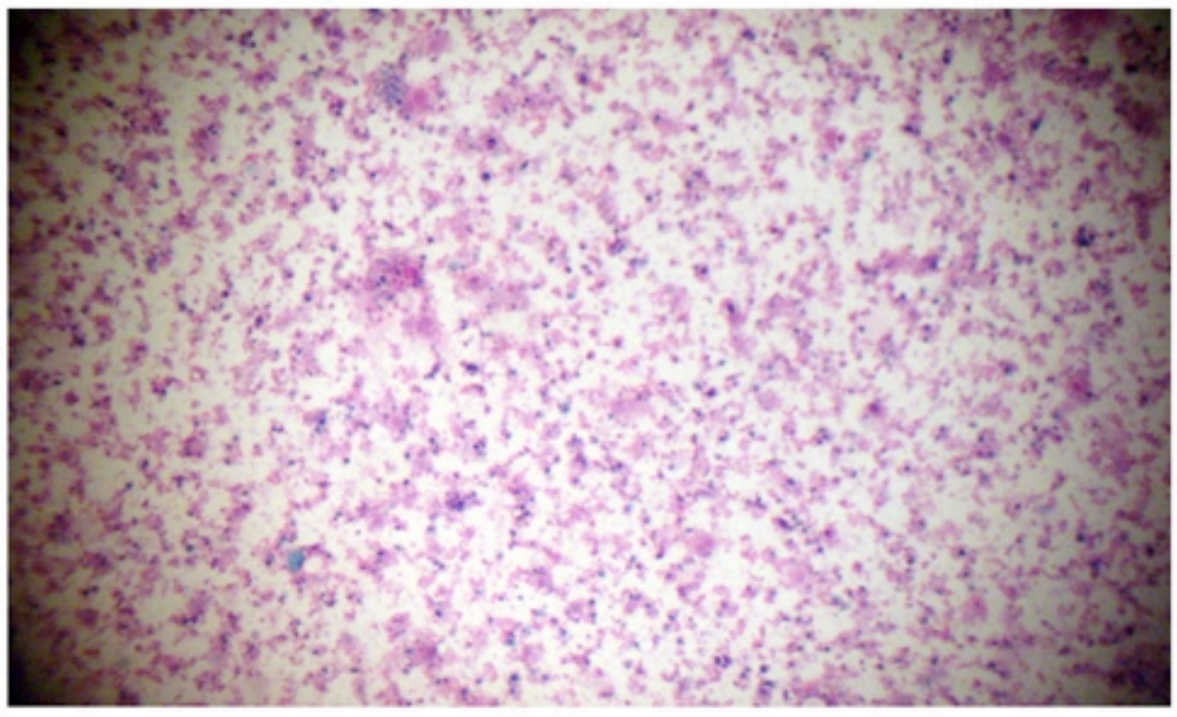
Ovarian Preculture
400X



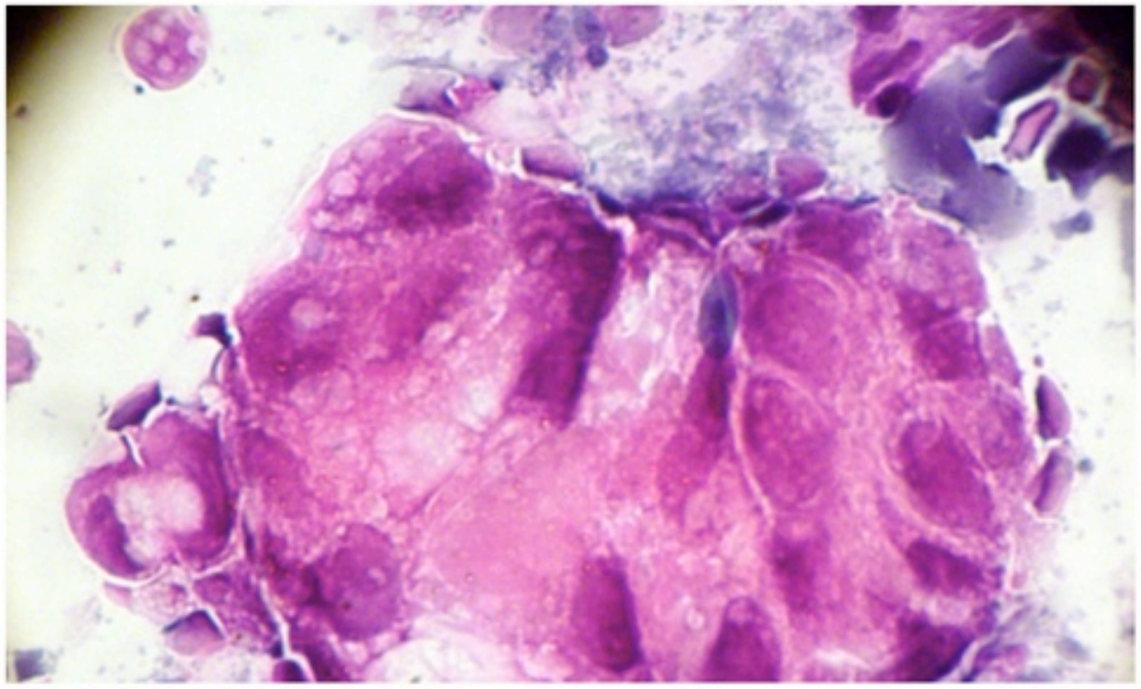
Postculture 40X



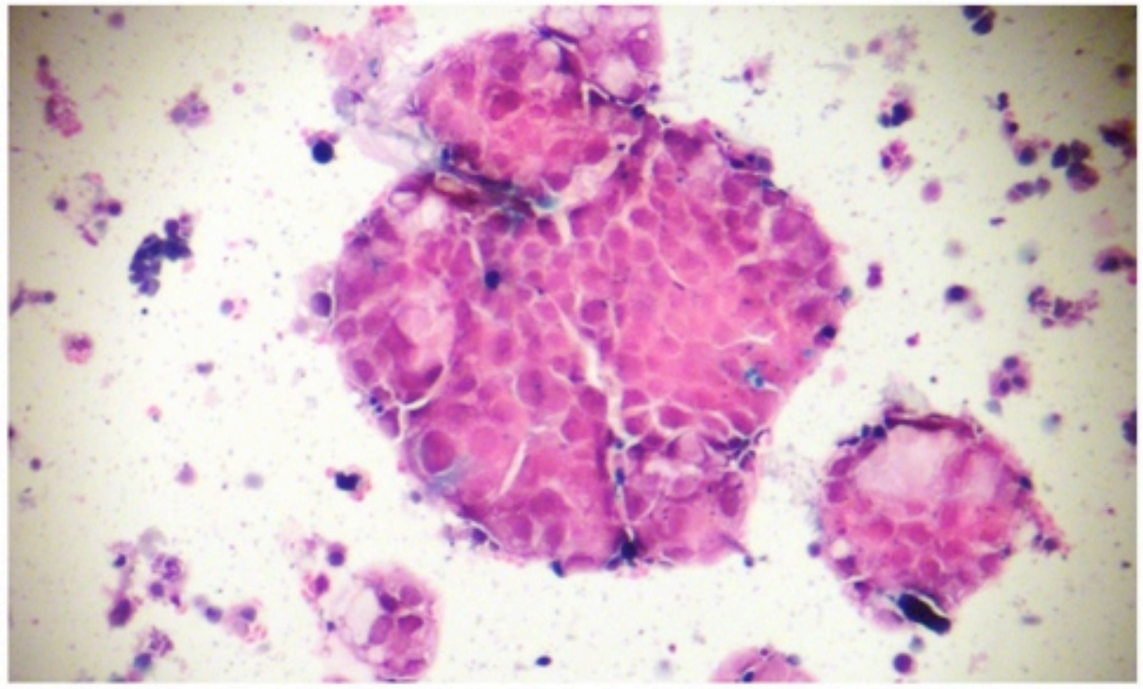
Doxil



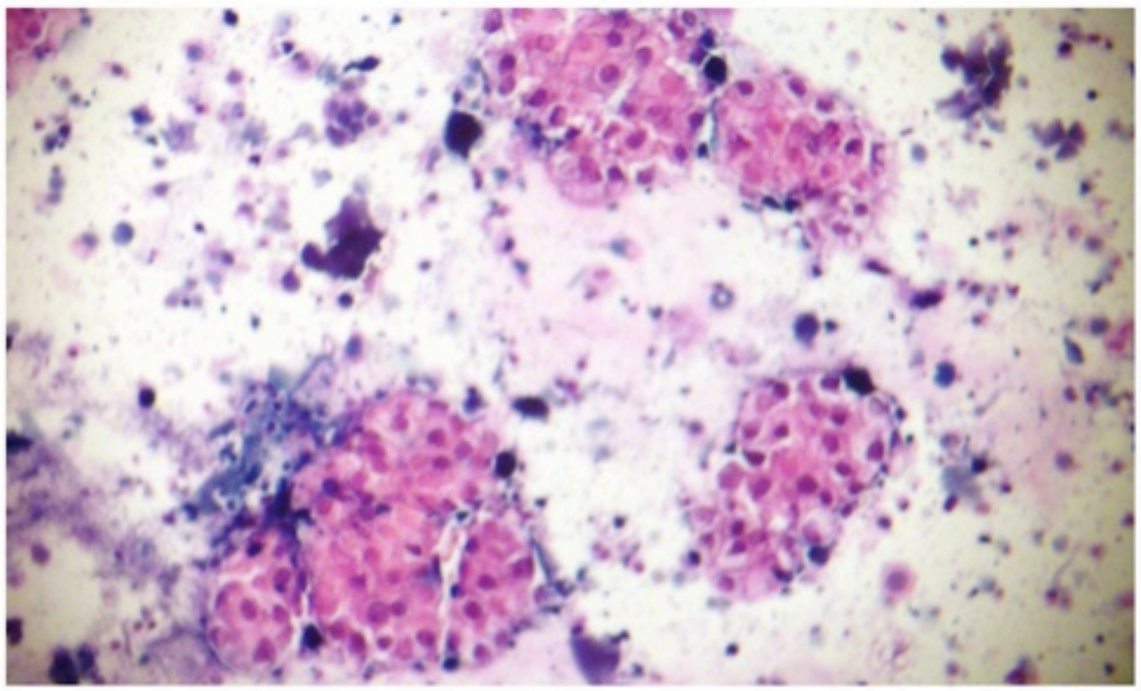
Topotecan



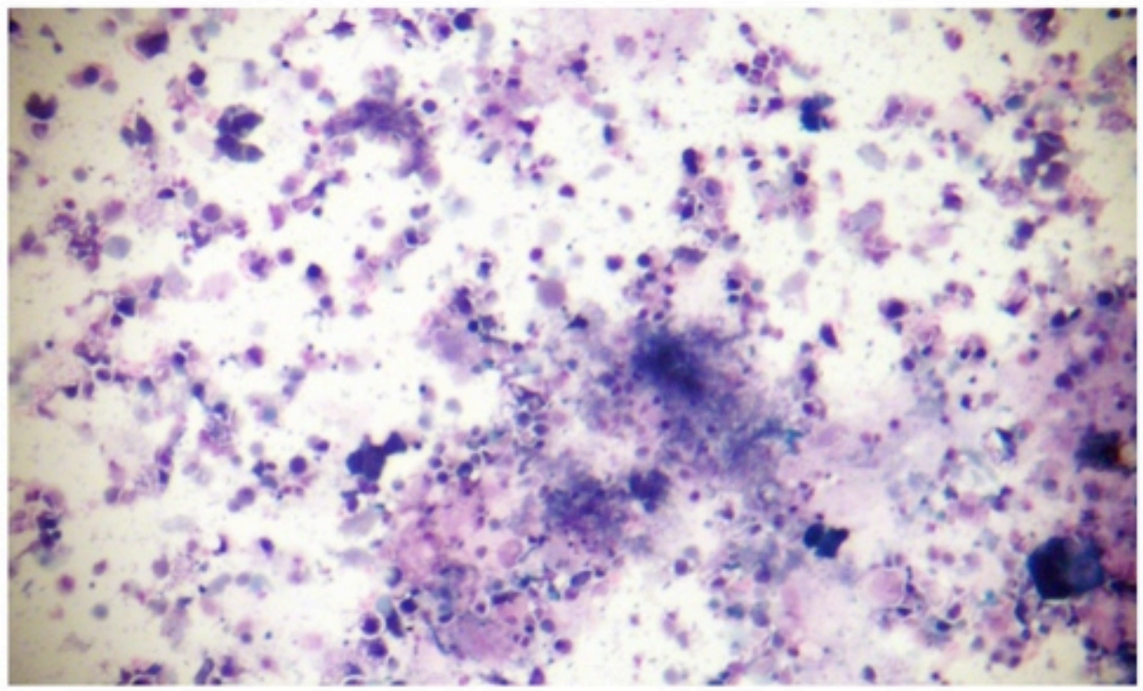
NSCLC Preculture
400X



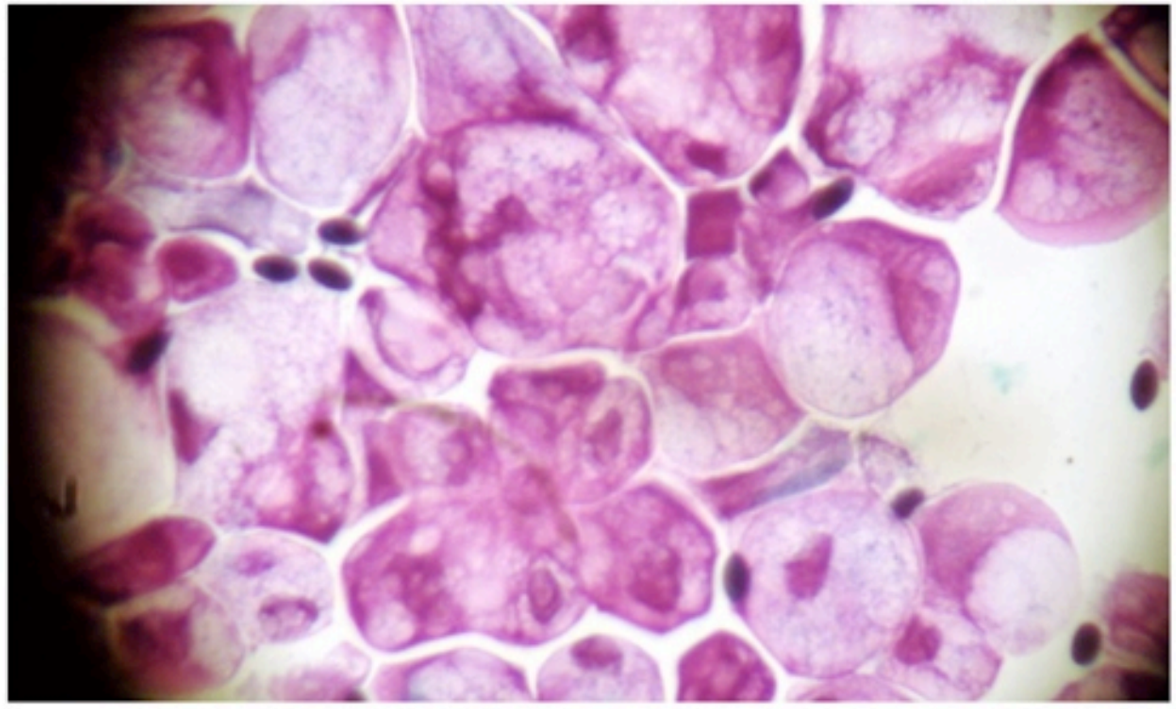
Postculture 100X



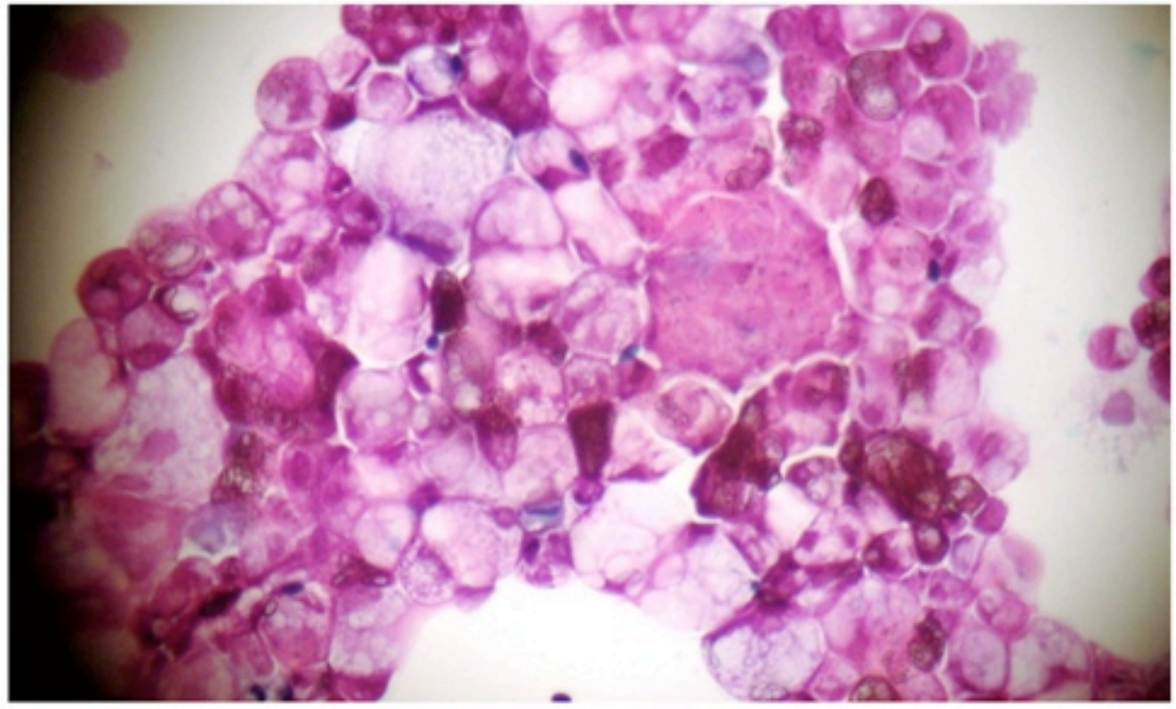
Docetaxel 100X



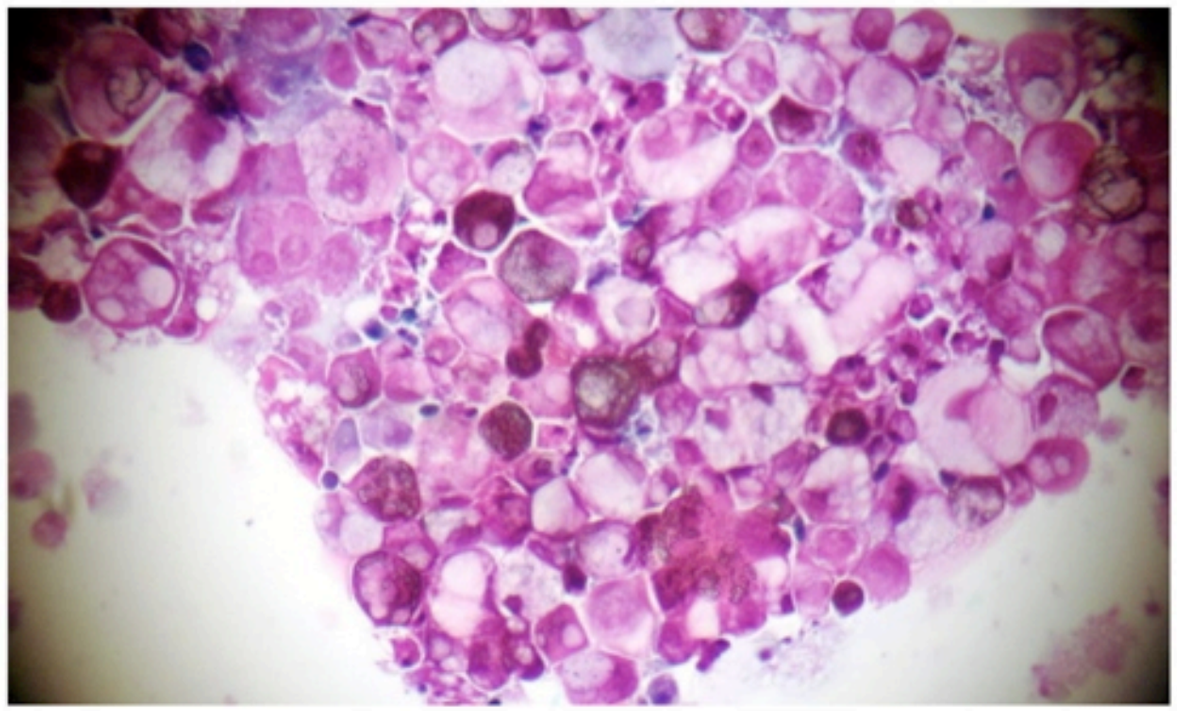
Cisplatin 100X



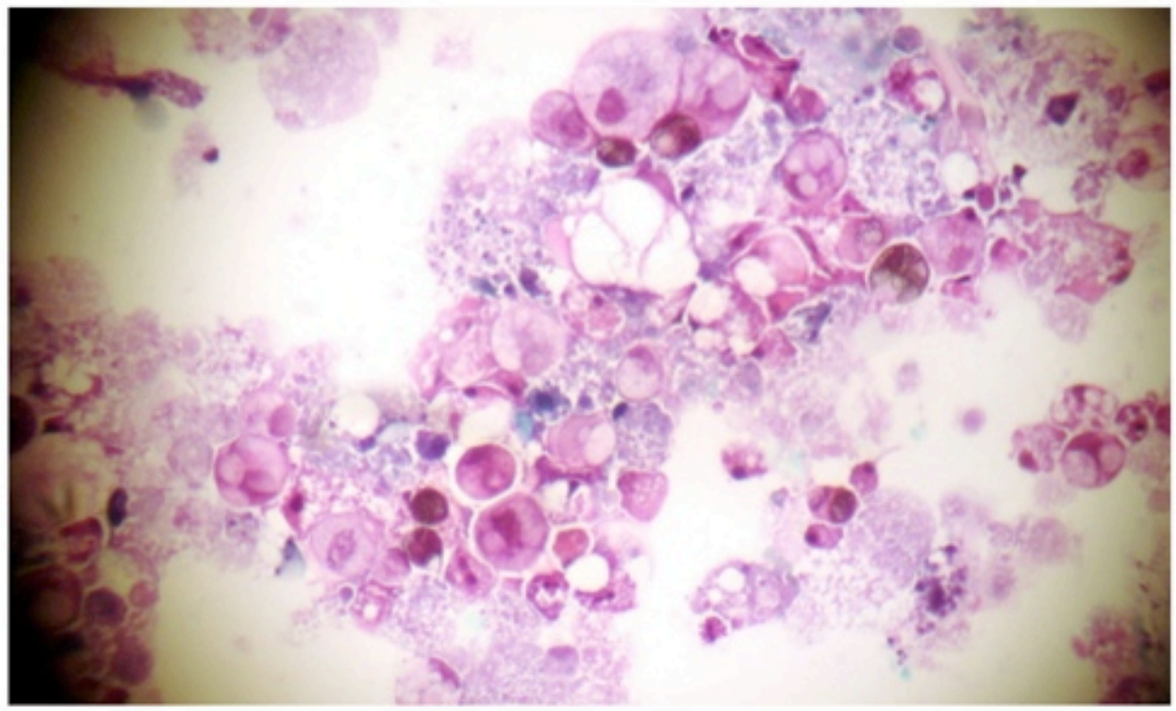
Pancreatic Preculture
400X



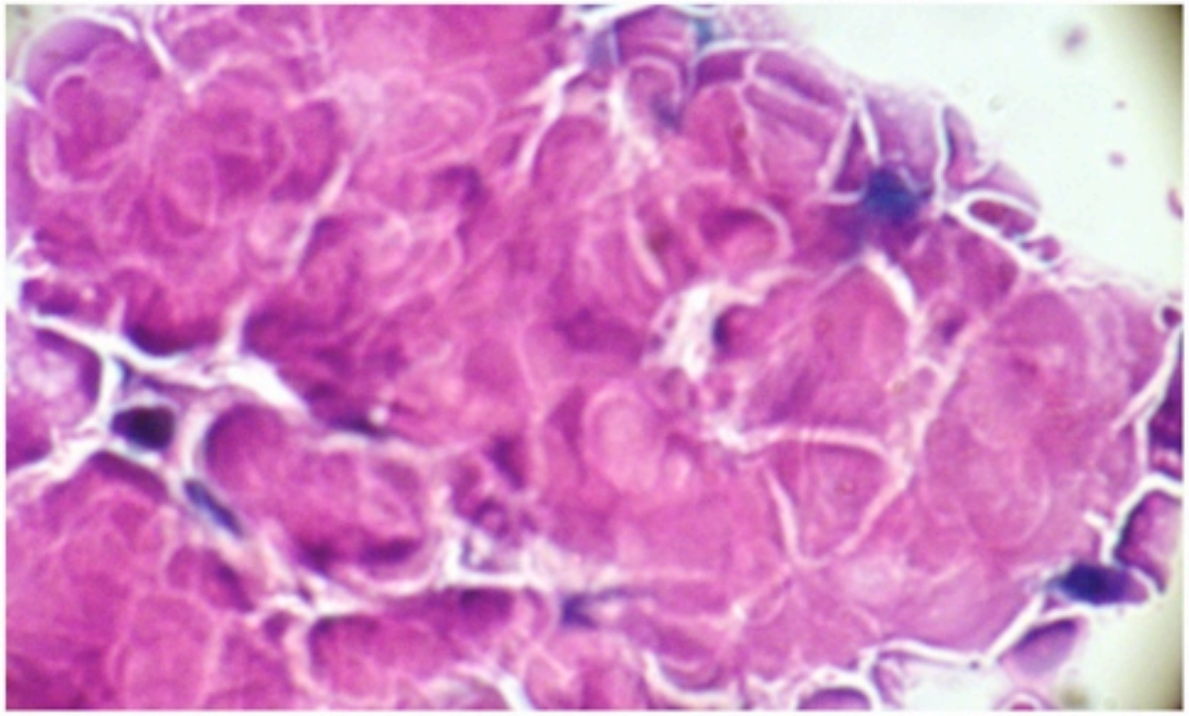
Postculture 200X



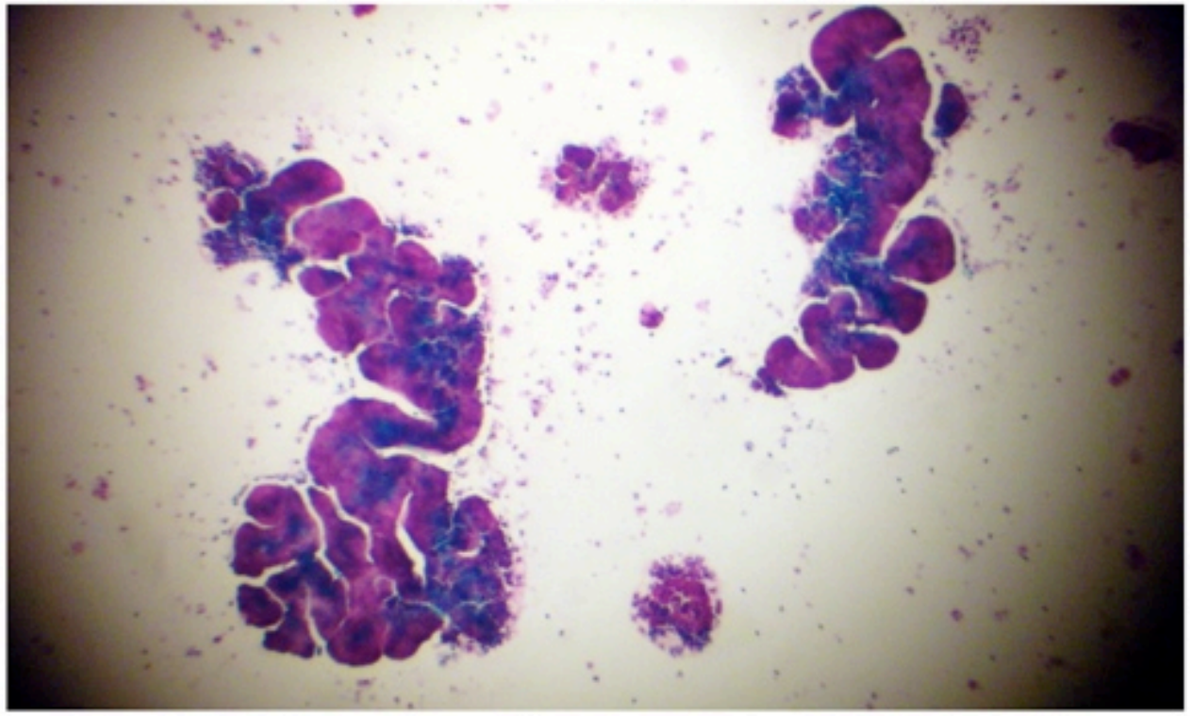
Cisplatin



Gemcitabine+Cisplatin



Colon Cancer Preculture
400X



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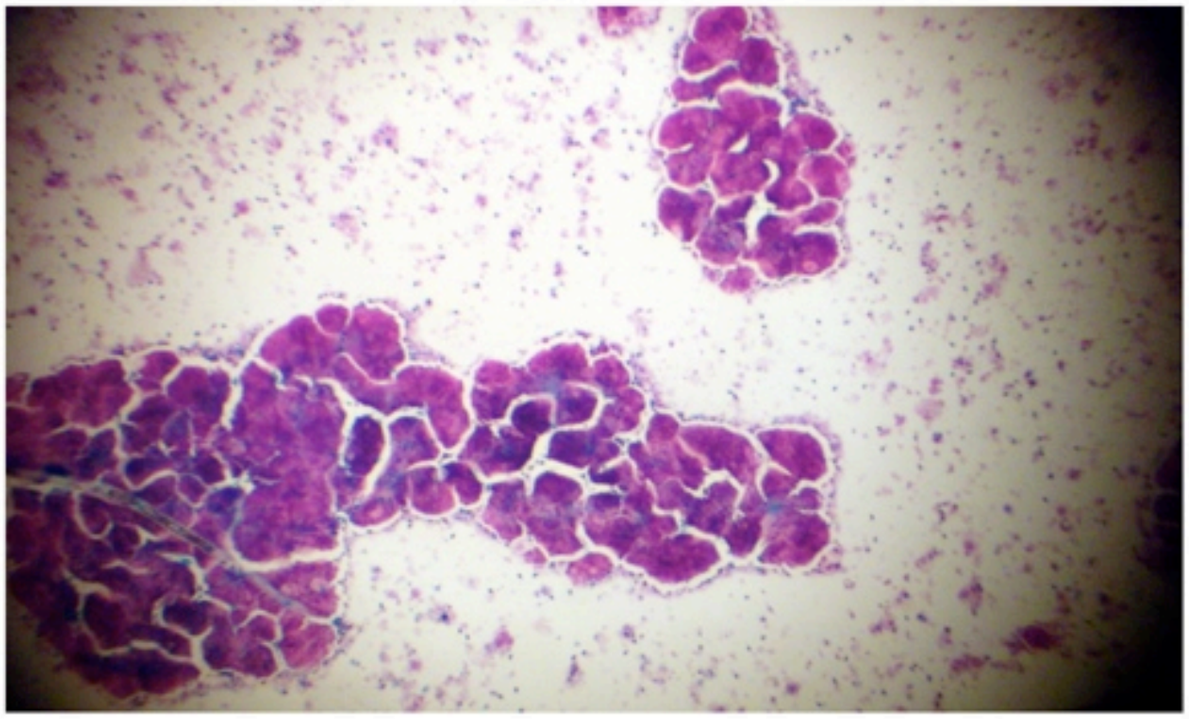
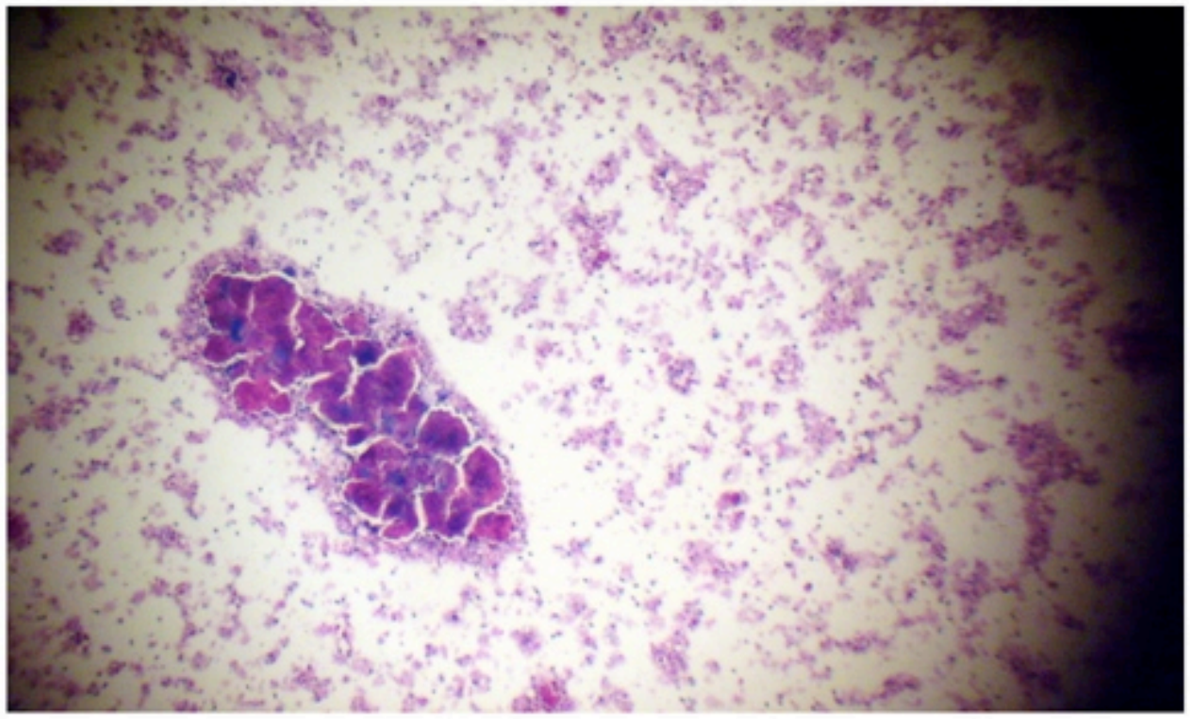
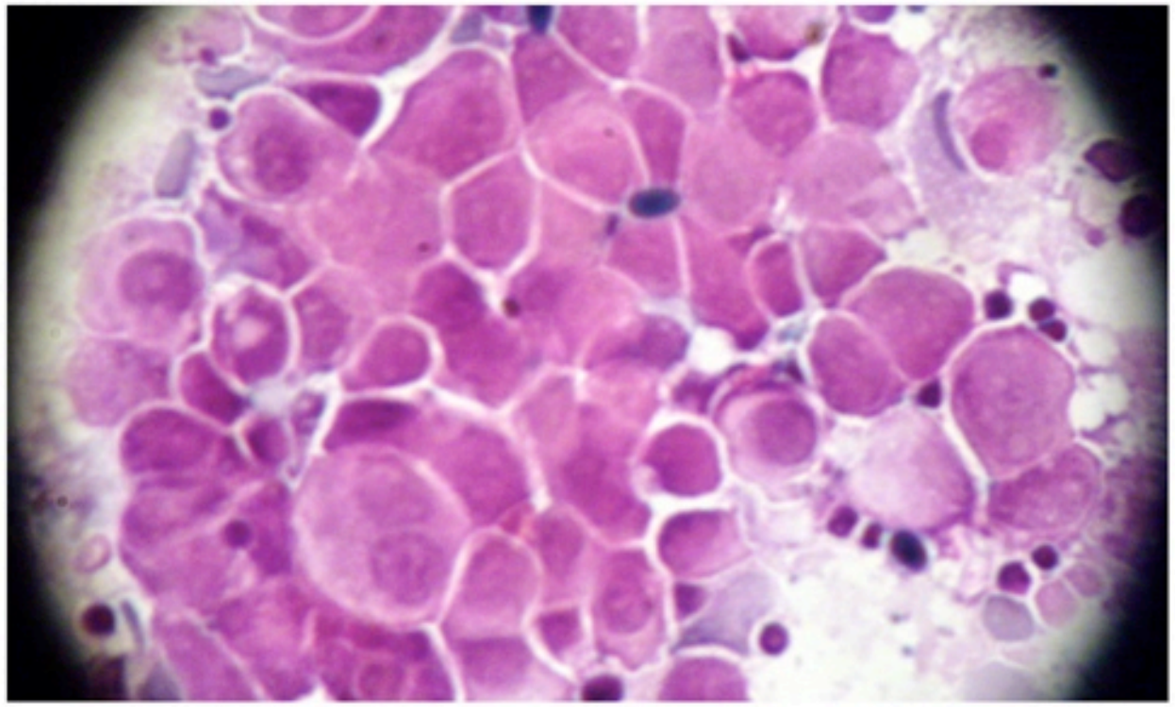


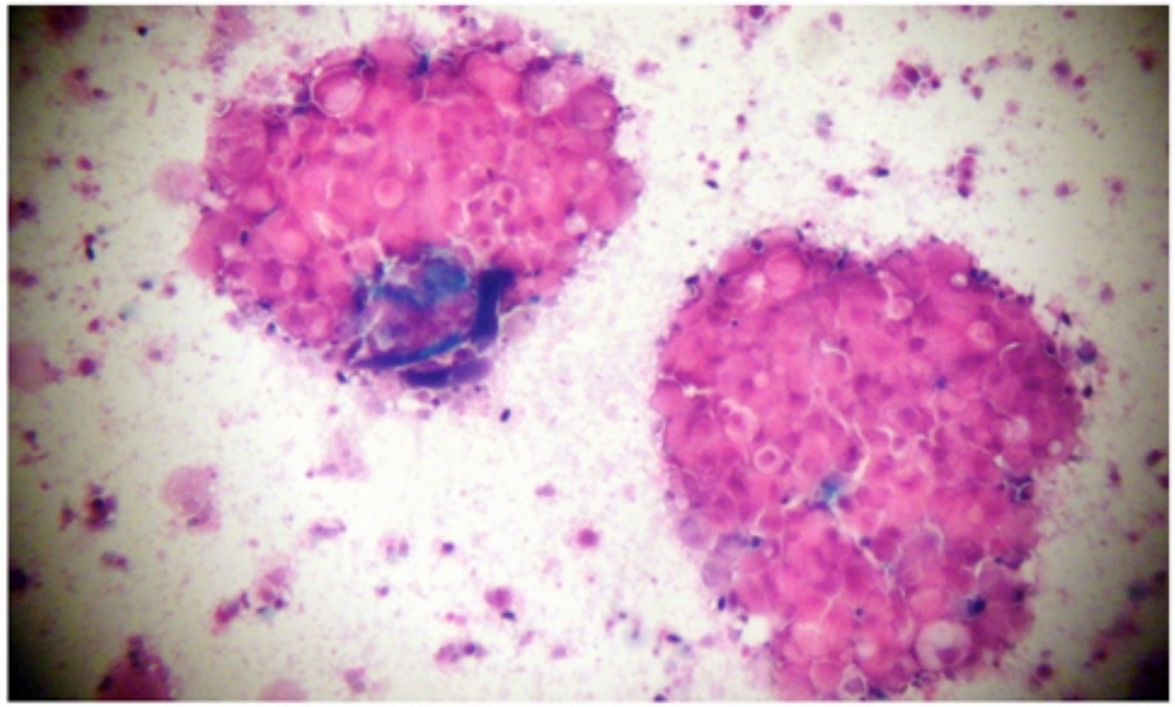
Oxaliplatin



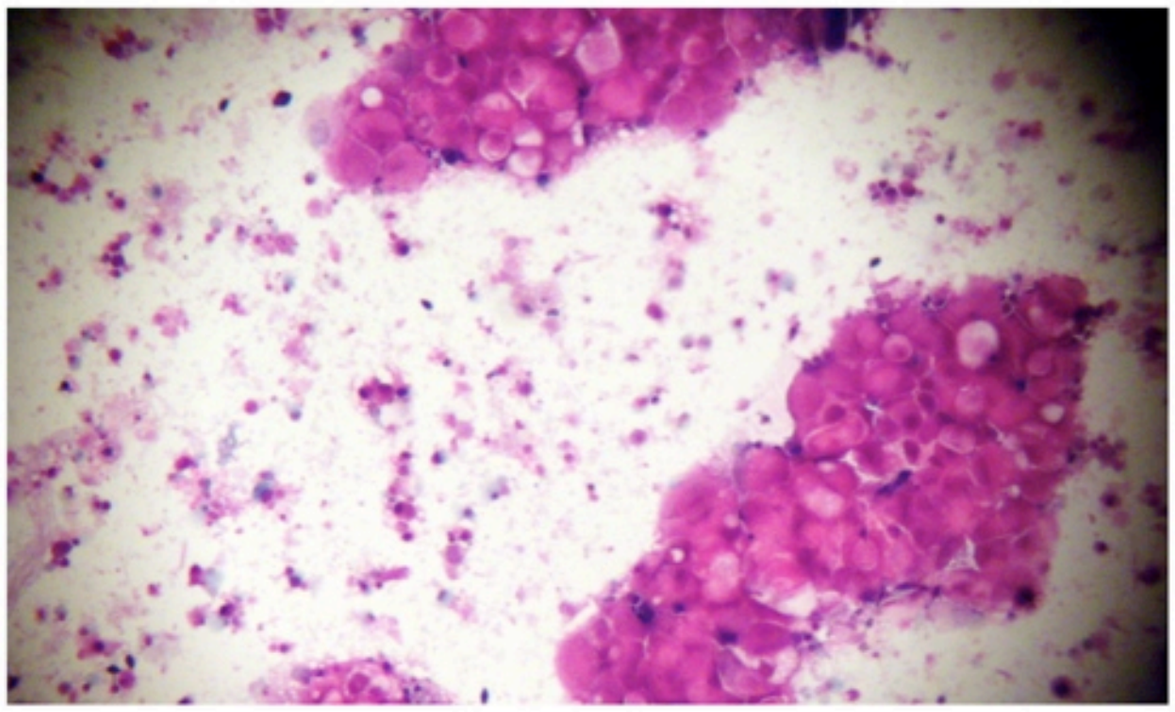
Gemcitabine+Oxaliplatin



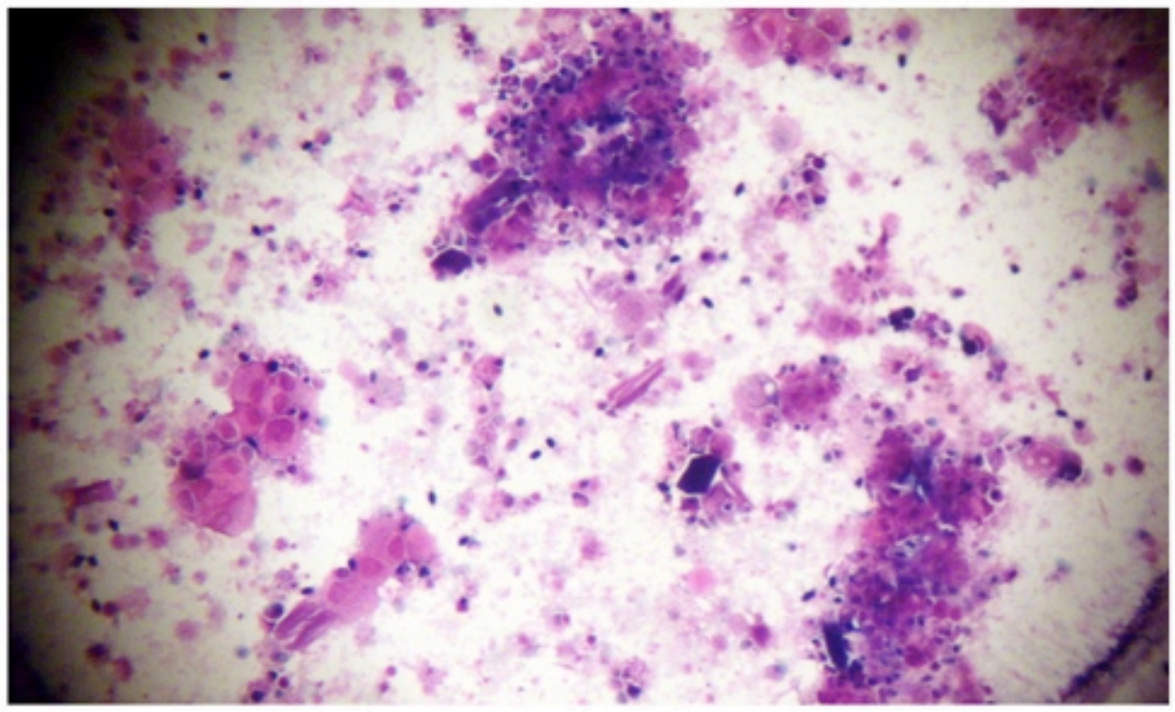
Breast Cancer Preculture
400X



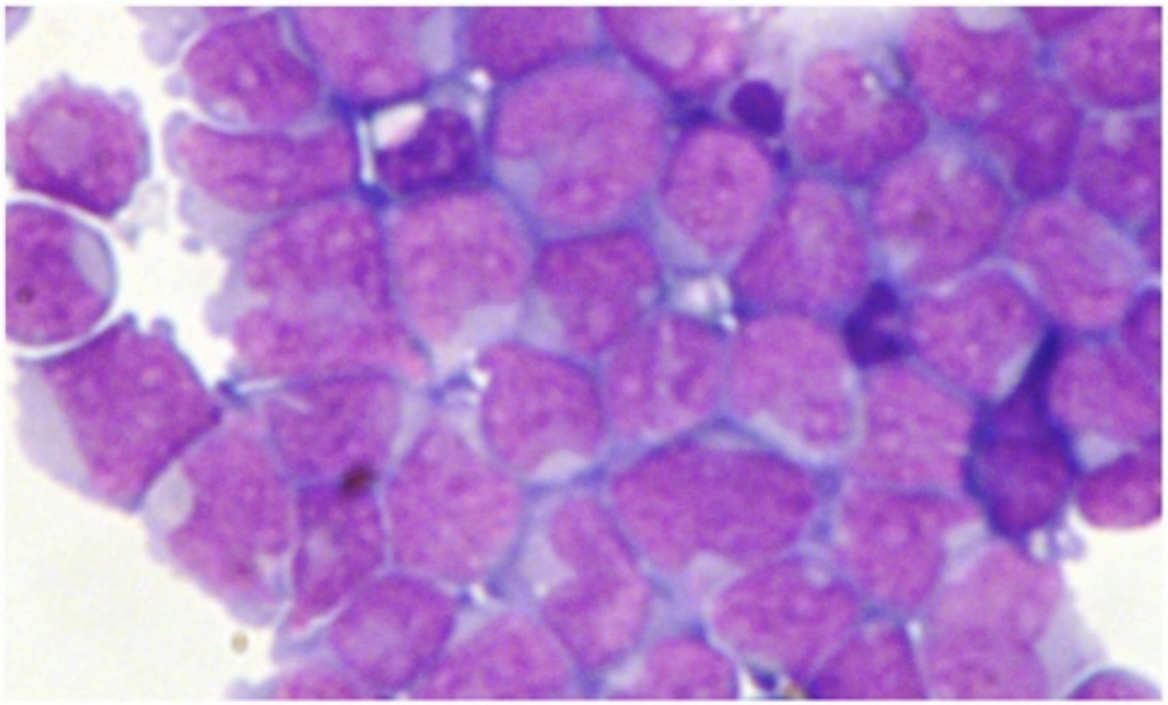
Postculture 100X



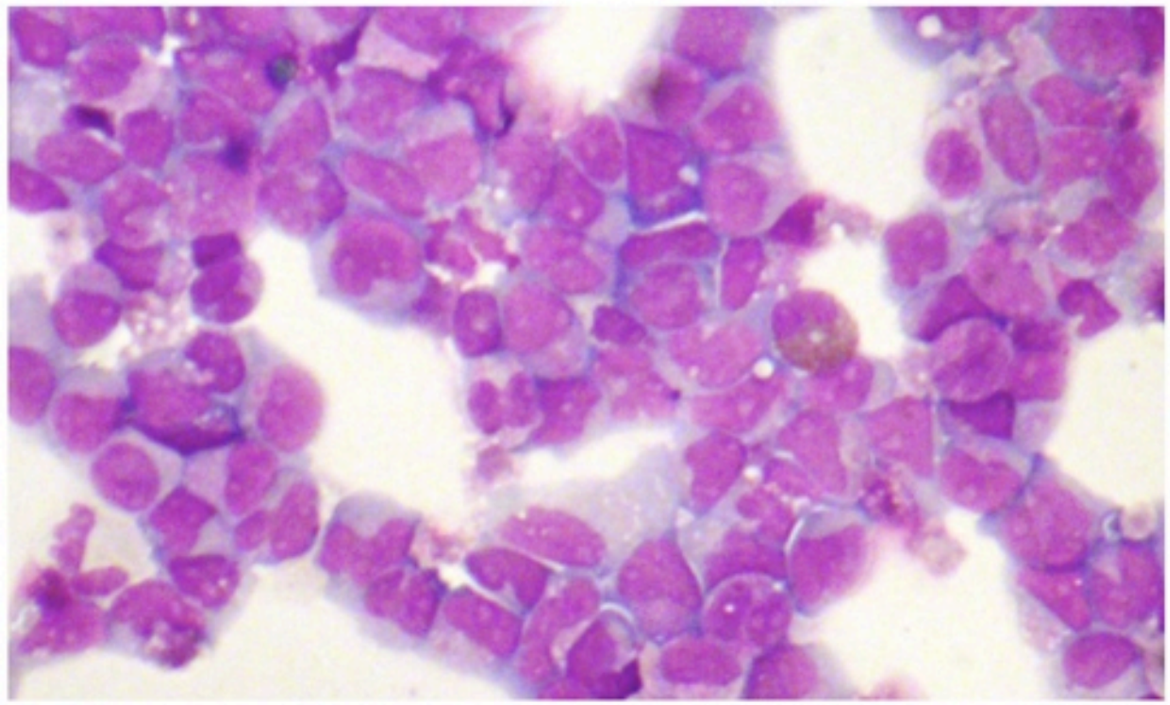
Cyclophosphamide (4HC)



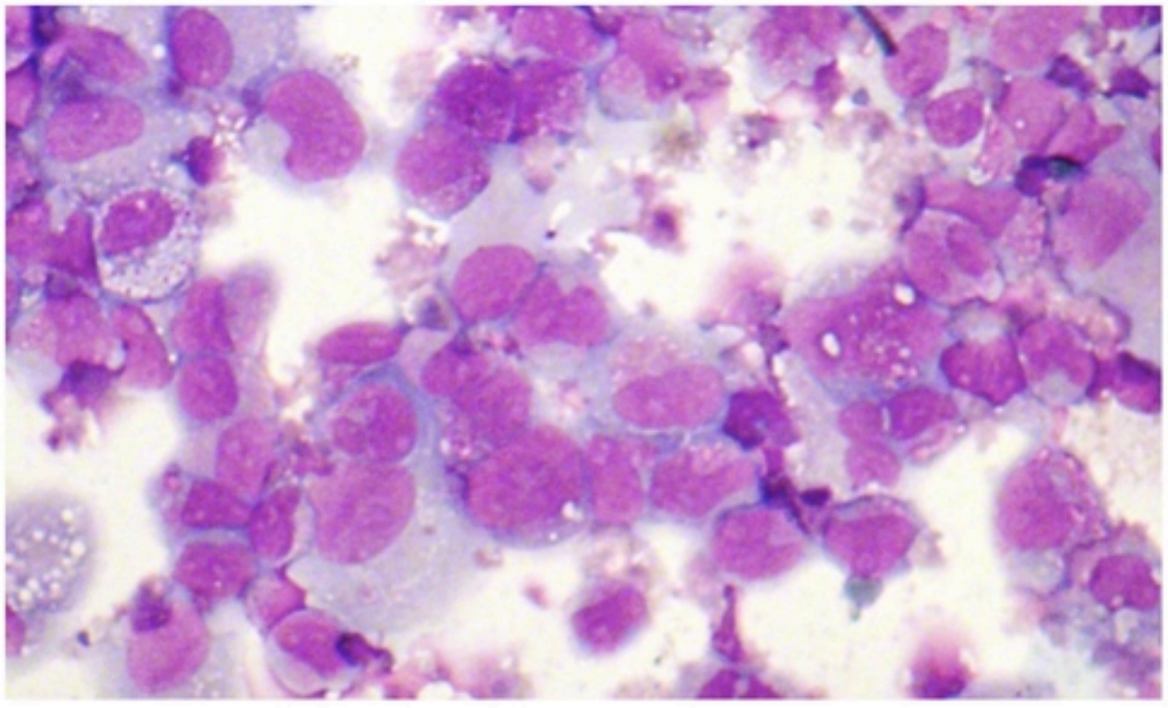
Vinorelbine+Tamoxifen



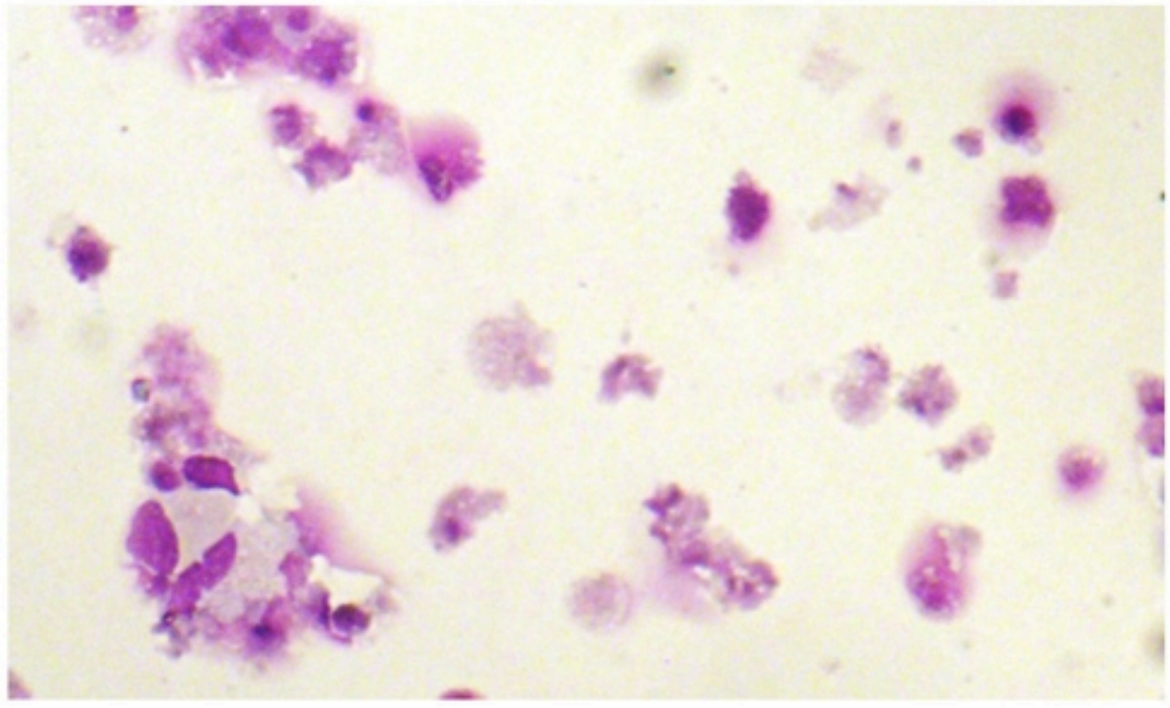
Non-Hodgkin's Preculture
400X



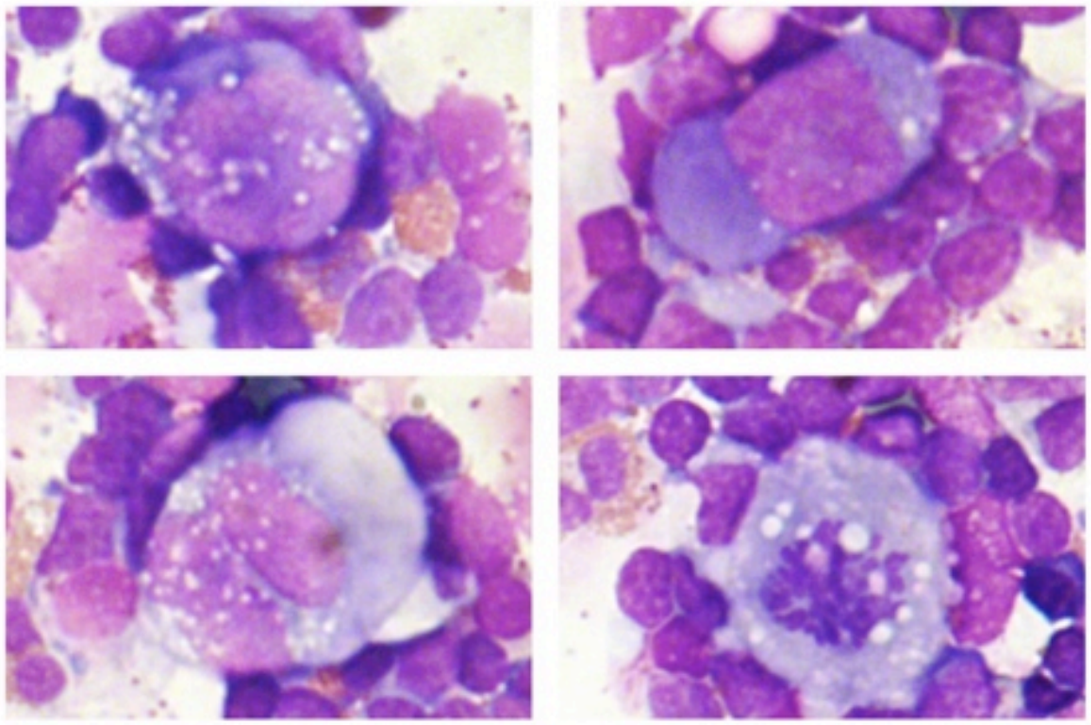
Postculture 200X



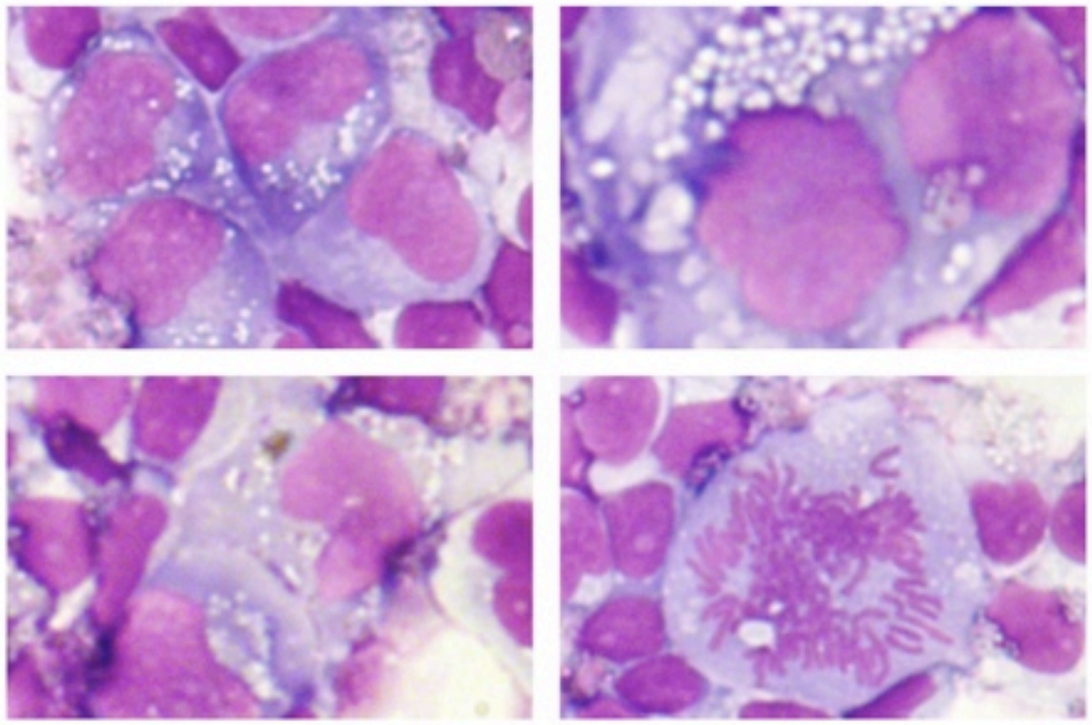
Doxorubicin



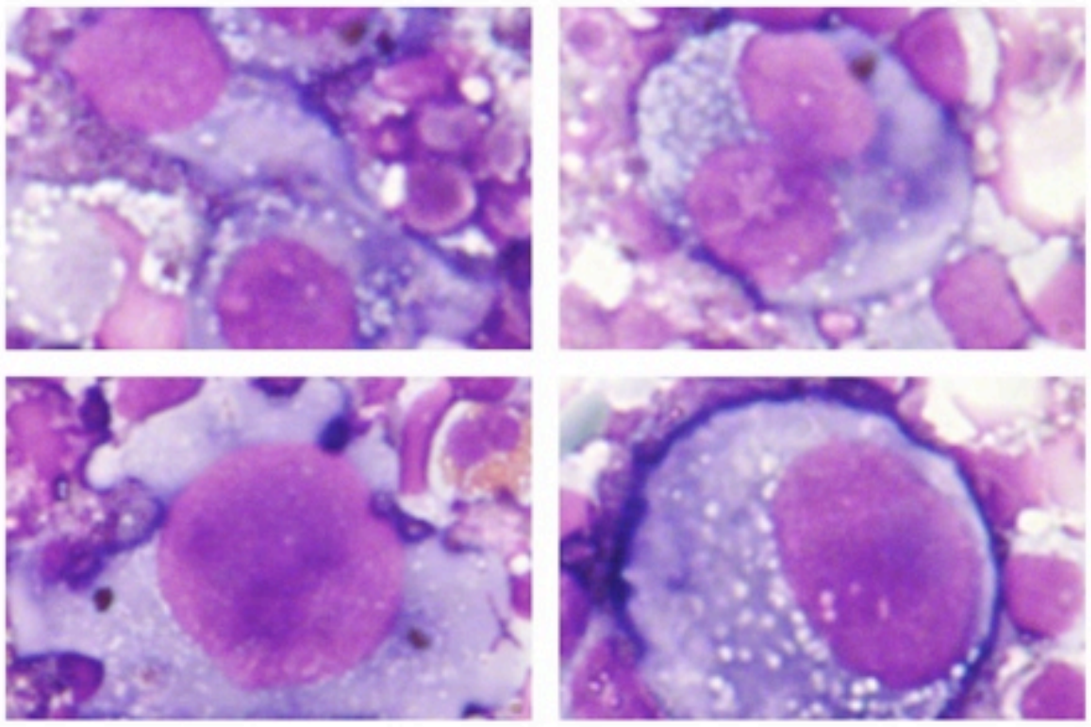
Fludarabine



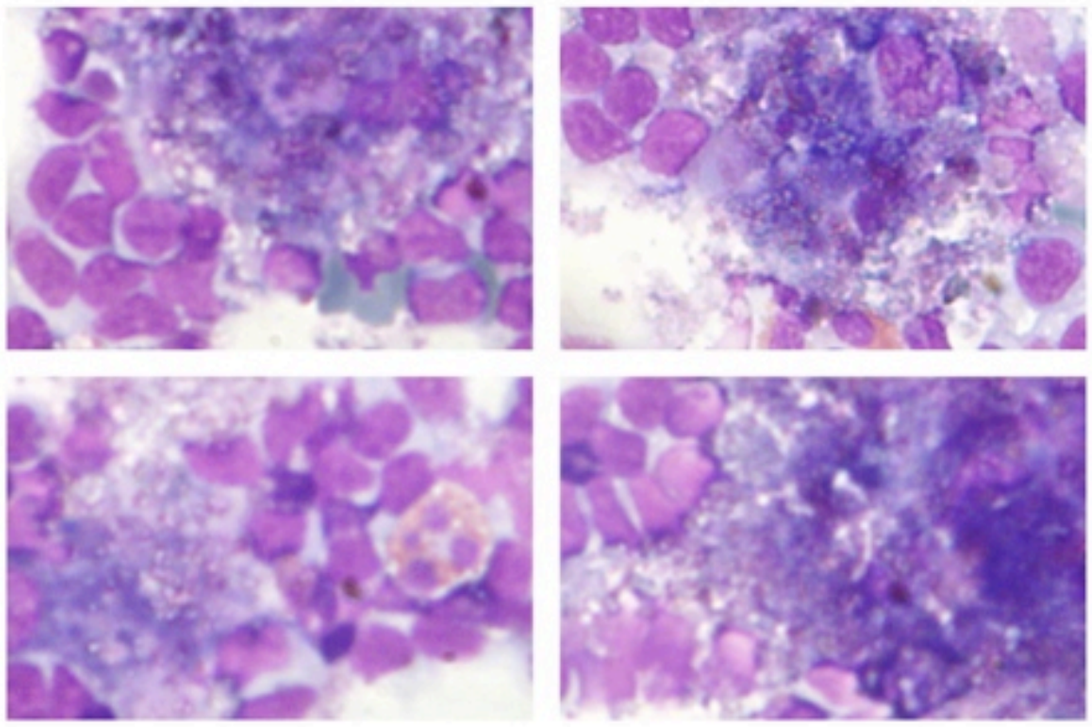
Hodgkin's Preculture



Hodgkin's Postculture

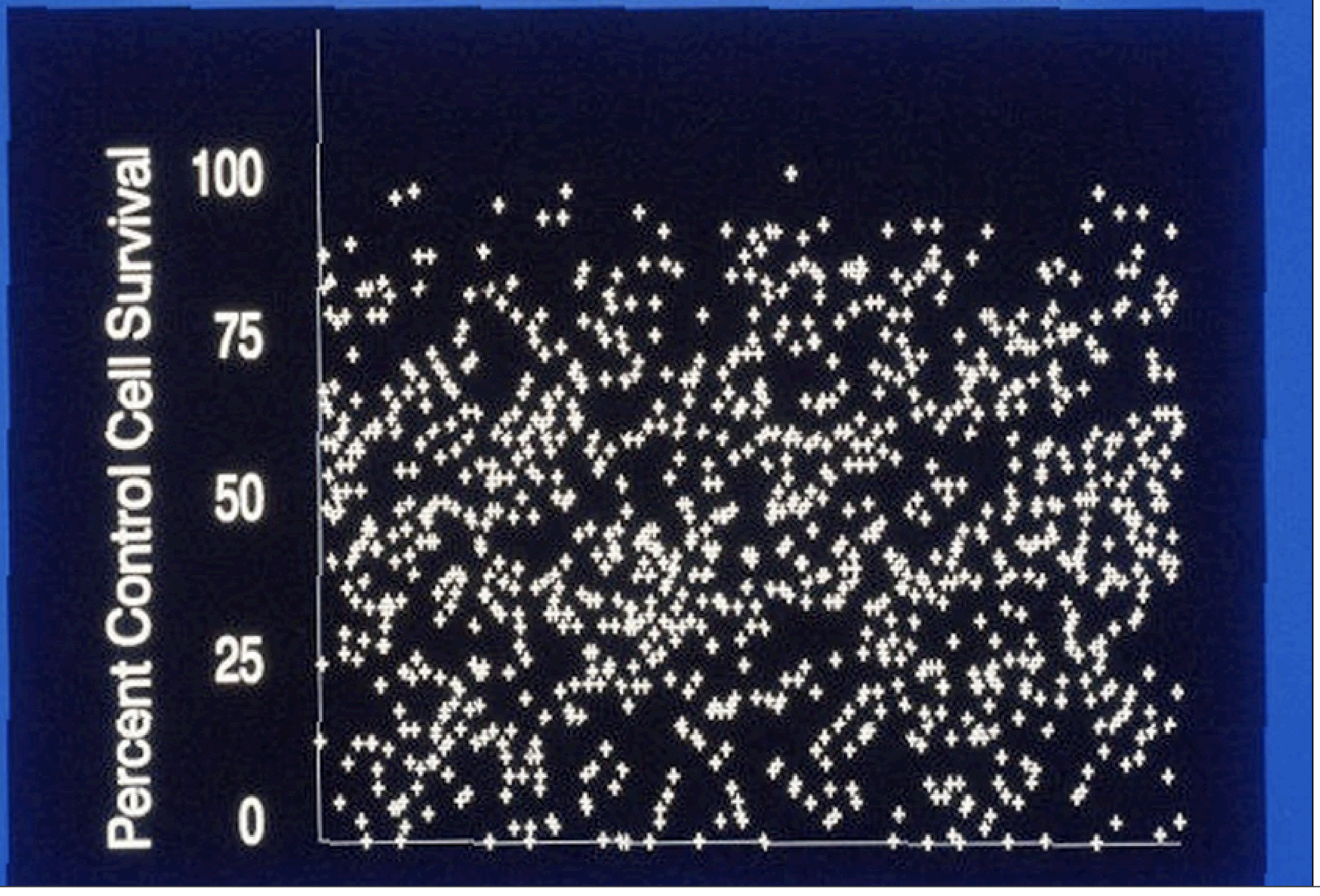


Cyclophosphamide (4HC)

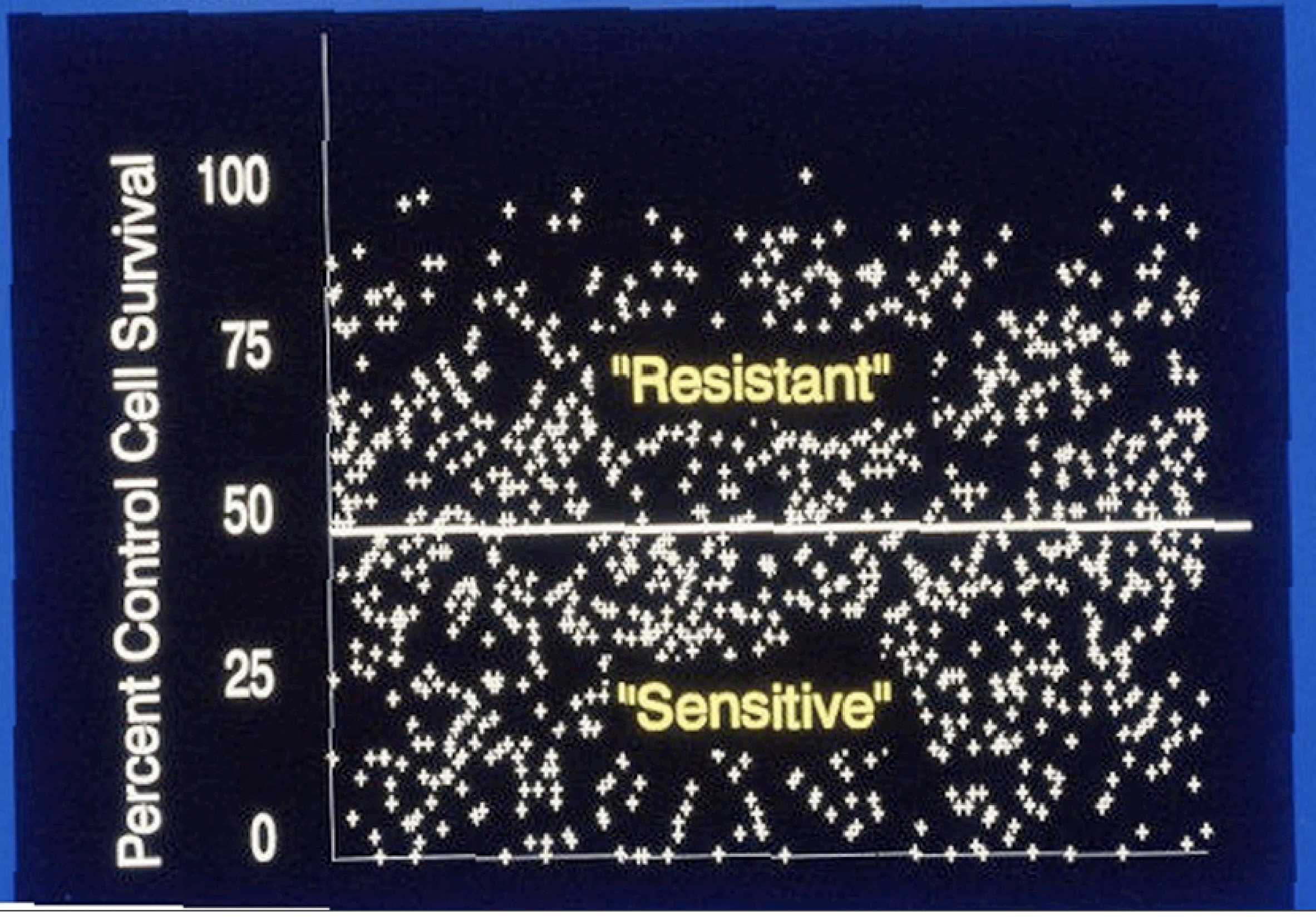


Irinotecan

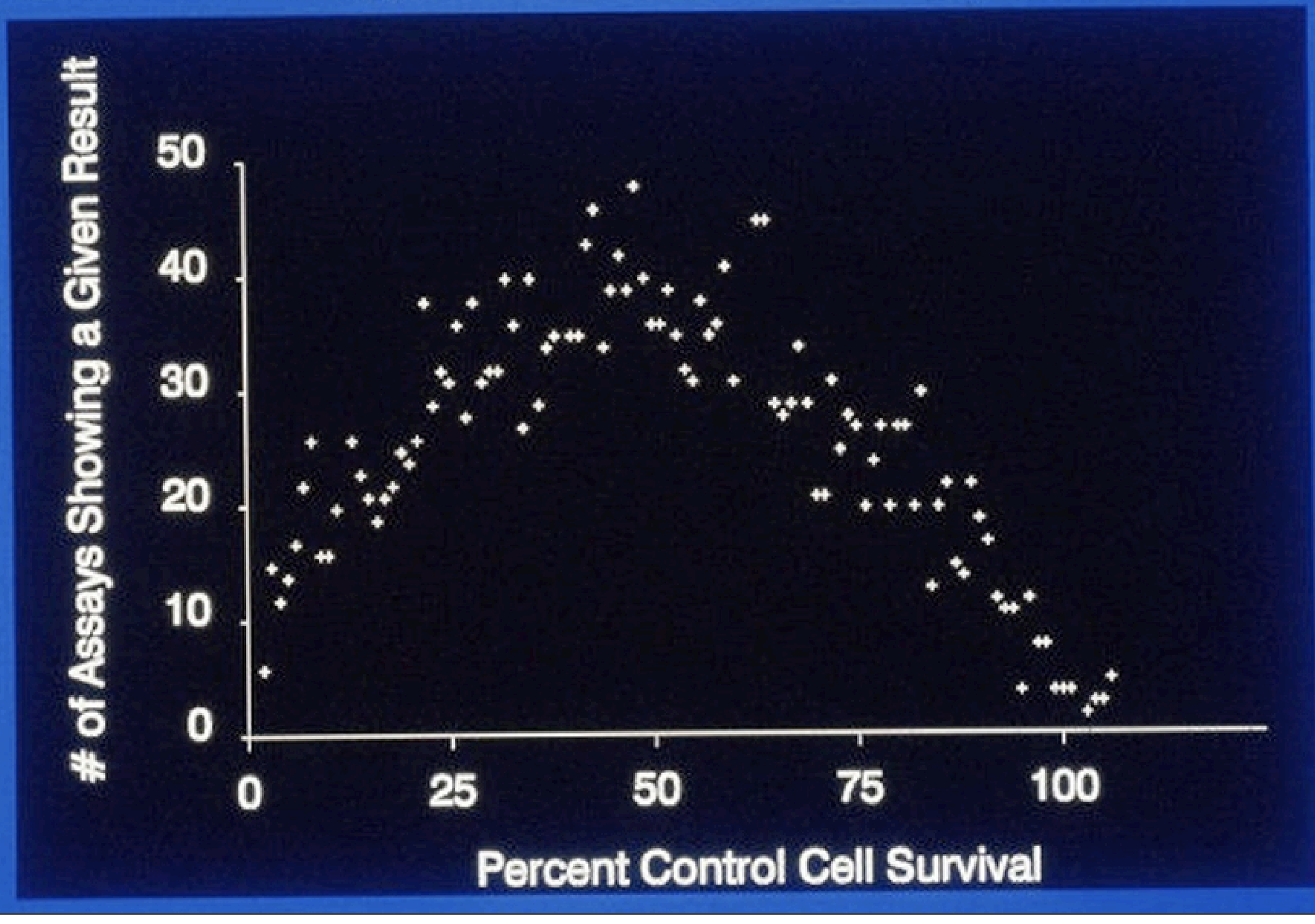
Cisplatin Activity in 1,000 Randomly-Selected Fresh Tumor MTT Assays



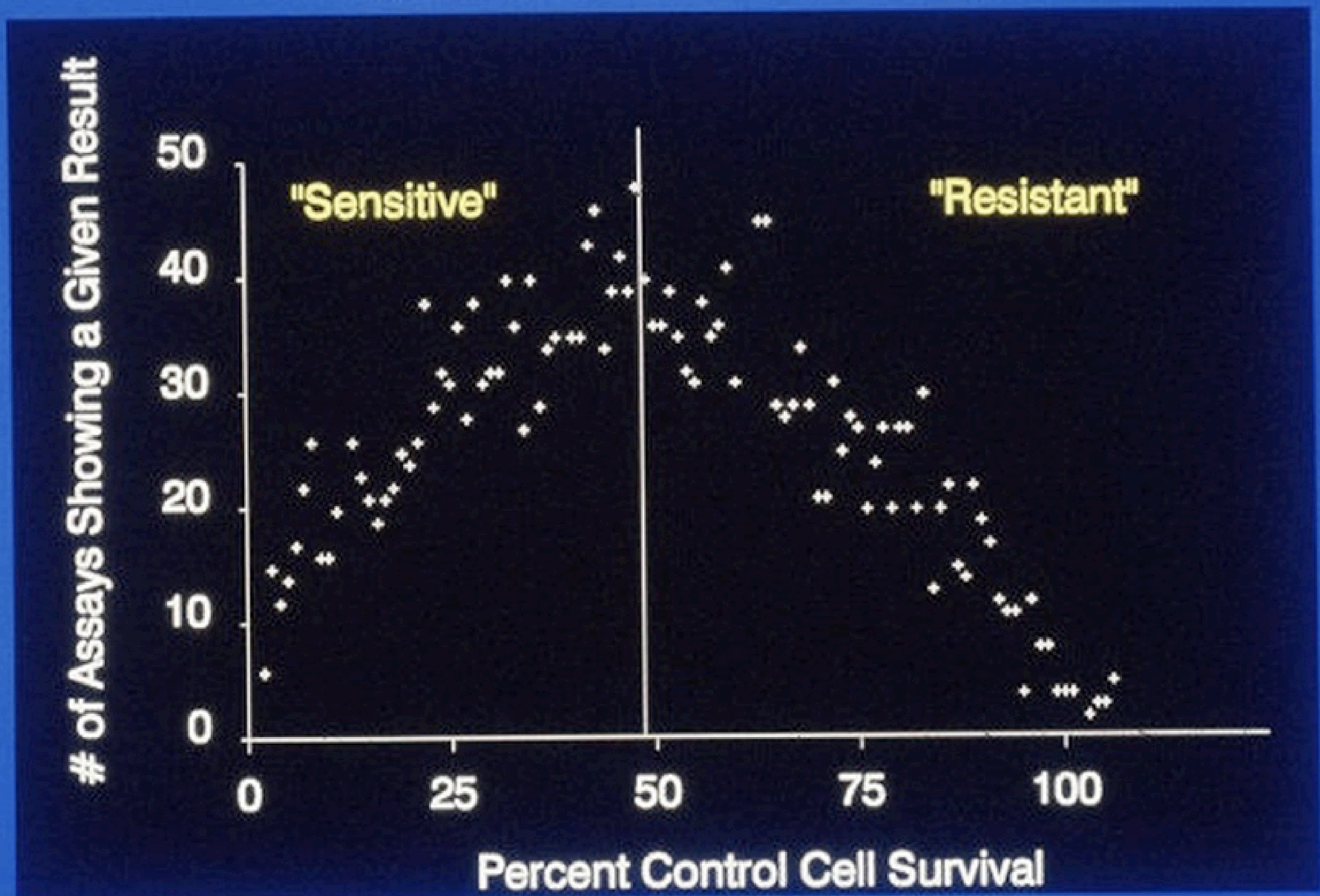
Cisplatin Activity in 1,000 Randomly-Selected Fresh Tumor MTT Assays



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



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



ASSAY REPORT

CLIA Lic. 05D0662326; CA Lab 203076; Medicare 558864

Laboratory Director: Larry M. Weisenthal MD, PhD

Weisenthal Cancer Group

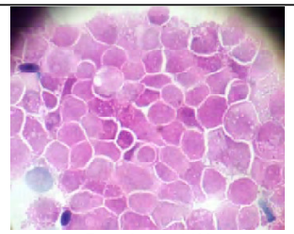
Patient and Physician

Name:		**DOB**	02/05/1946	**I.D.**	H3126
M.D.		**Dx.**	Breast Cancer	**Prior Rx.**	CMF; CTX/ Docetaxel/5FU; Vinorelbine

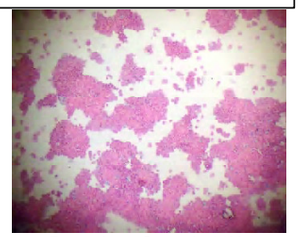
Specimen

Collected:	03/14/07	**Path. Accession No.:**	VVM-07-1570
Received:	03/15/07	**Specimen Site:**	Fluid,Pleural
Reported:	03/28/07		

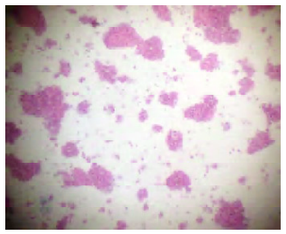
Specimen Quality Factors On the photomicrographs shown, dead cells and chromatinaceous debris stain blue; living cells stain red. Please see also assay description and "targeted" assay report.



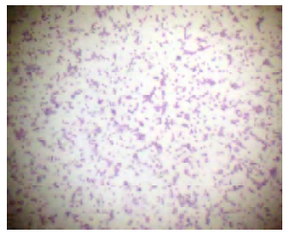
Preculture 400X



Postculture Control 100X



Gemcitabine/Cisplatin 100X



Treosulfan 100X

Testing

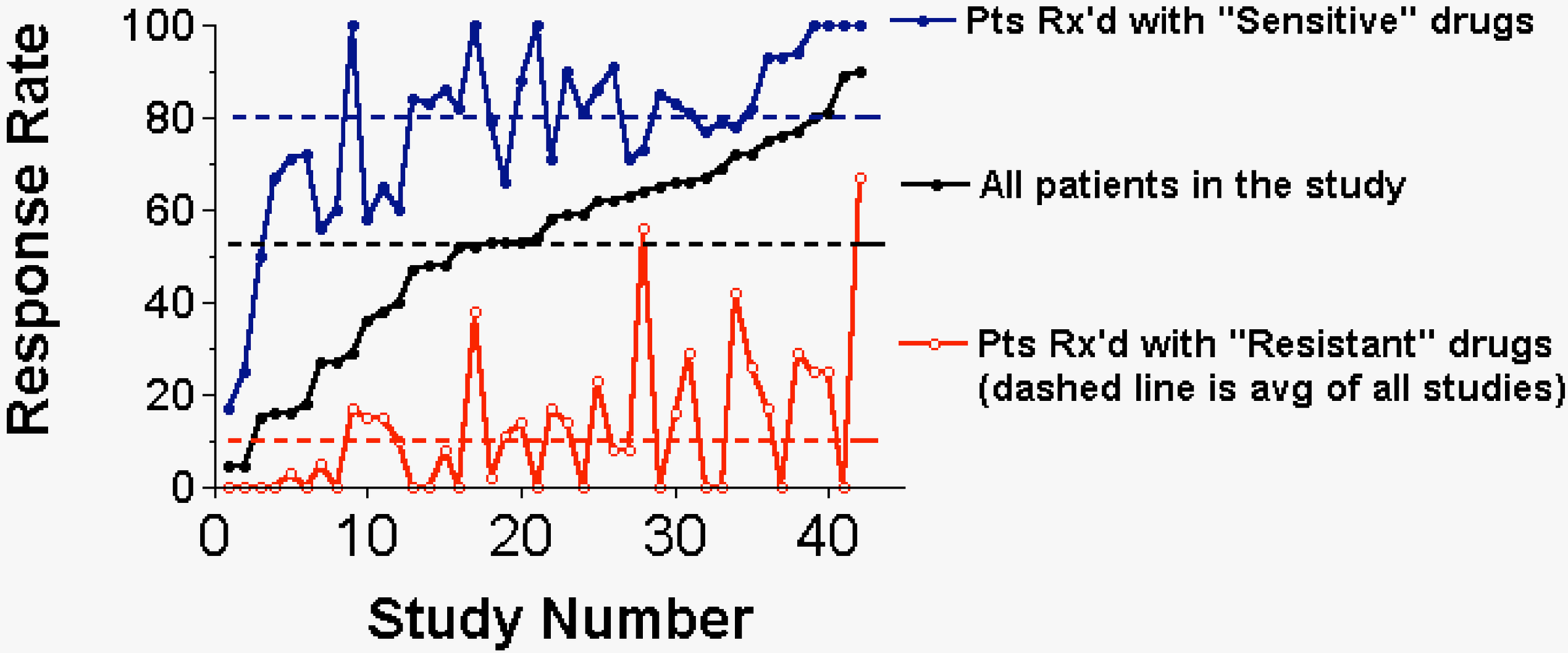
Tests	Agents	Combinations	Technical Quality
DiSC	23	4	Good
MTT	20	6	Good
ATP	0	0	N/A
Caspase 3/7	0	0	N/A

Comments

Assay Results

Agents	Assay Results Qualitative	Assay Predicted Response Probability	Reference Range (Literature Response Rate)
Cisplatin	*Resistant*	0.03	0.15
Oxaliplatin	Intermediate	0.15	0.15
Cyclophosphamide(4HC)	Intermediate	0.15	0.15
Treosulfan	**Sensitive**	0.38	0.15
Docetaxel (Taxotere)	Intermediate	0.15	0.15
Docetaxel+Epirubicin	Intermediate	0.20	0.20
Paclitaxel (Taxol)	*Resistant*	0.03	0.15
Doxorubicin	Intermediate	0.15	0.15
Etoposide	*Resistant*	0.03	0.15
Floxuridine	*Resistant*	0.03	0.15
Fluorouracil	*Resistant*	0.03	0.15
Fluorouracil+Leucovorin	*Resistant*	0.03	0.15
Gemcitabine (Gemzar)	*Resistant*	0.03	0.15
Gemcitabine+Cisplatin	Intermediate	0.25	0.25
Gemcitabine+Mitomycin C	**Sensitive**	0.47	0.20
Gemcitabine+Oxaliplatin	**Sensitive**	0.55	0.25
Mitomycin C	**Sensitive**	0.38	0.15
Vinorelbine (Navelbine)	**Sensitive**	0.38	0.15
Irinotecan (CPT-11)	*Resistant*	0.02	0.10
Irinotecan+Mitomycin C	**Sensitive**	0.47	0.20
Irinotecan+Oxaliplatin	**Sensitive**	0.47	0.20
Rubitecan (9-aminocamptothecin)	Intermediate	0.10	0.10
Topotecan (Hycamtin)	*Resistant*	0.02	0.10
Bortezomib (Velcade)	*Resistant*	0.02	0.10
Erlotinib+Sorafenib	*Resistant*	0.02	0.10

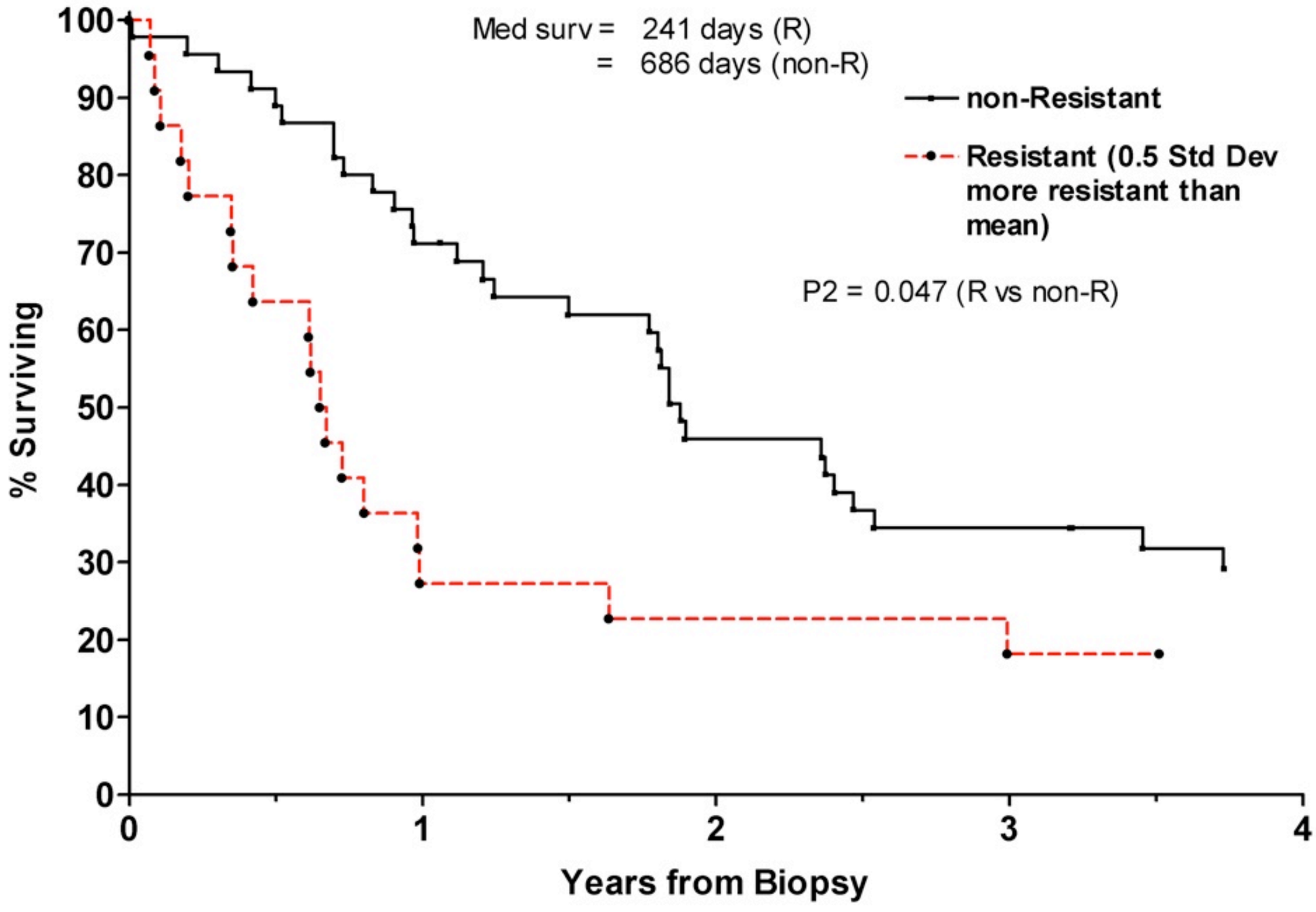
Correlation between LCA (cell death) results and clinical response to chemotherapy in 42 studies involving 1945 patients



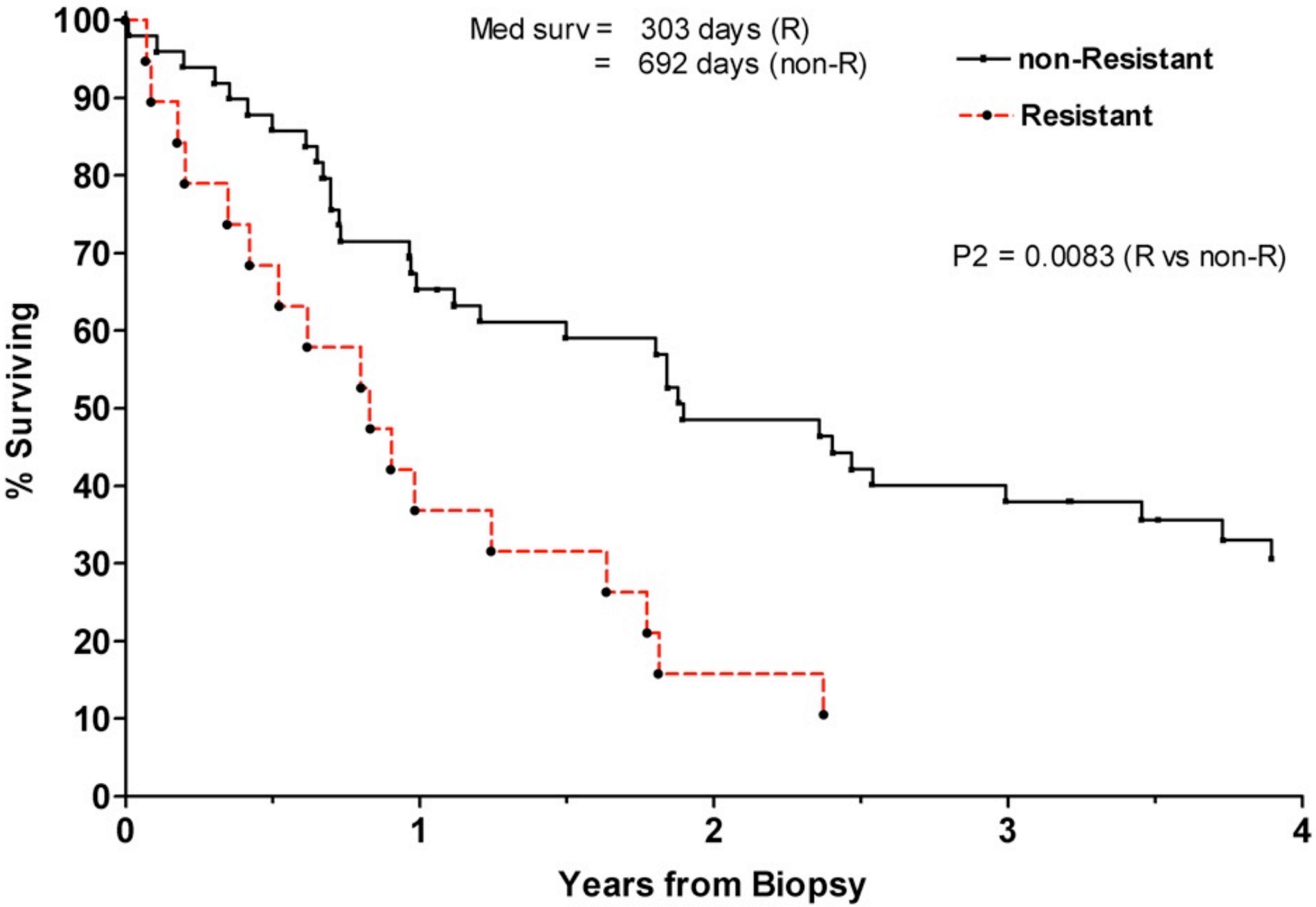
- There have been more than 25 peer review publications showing significant correlations between cell death assay results and patient survival

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

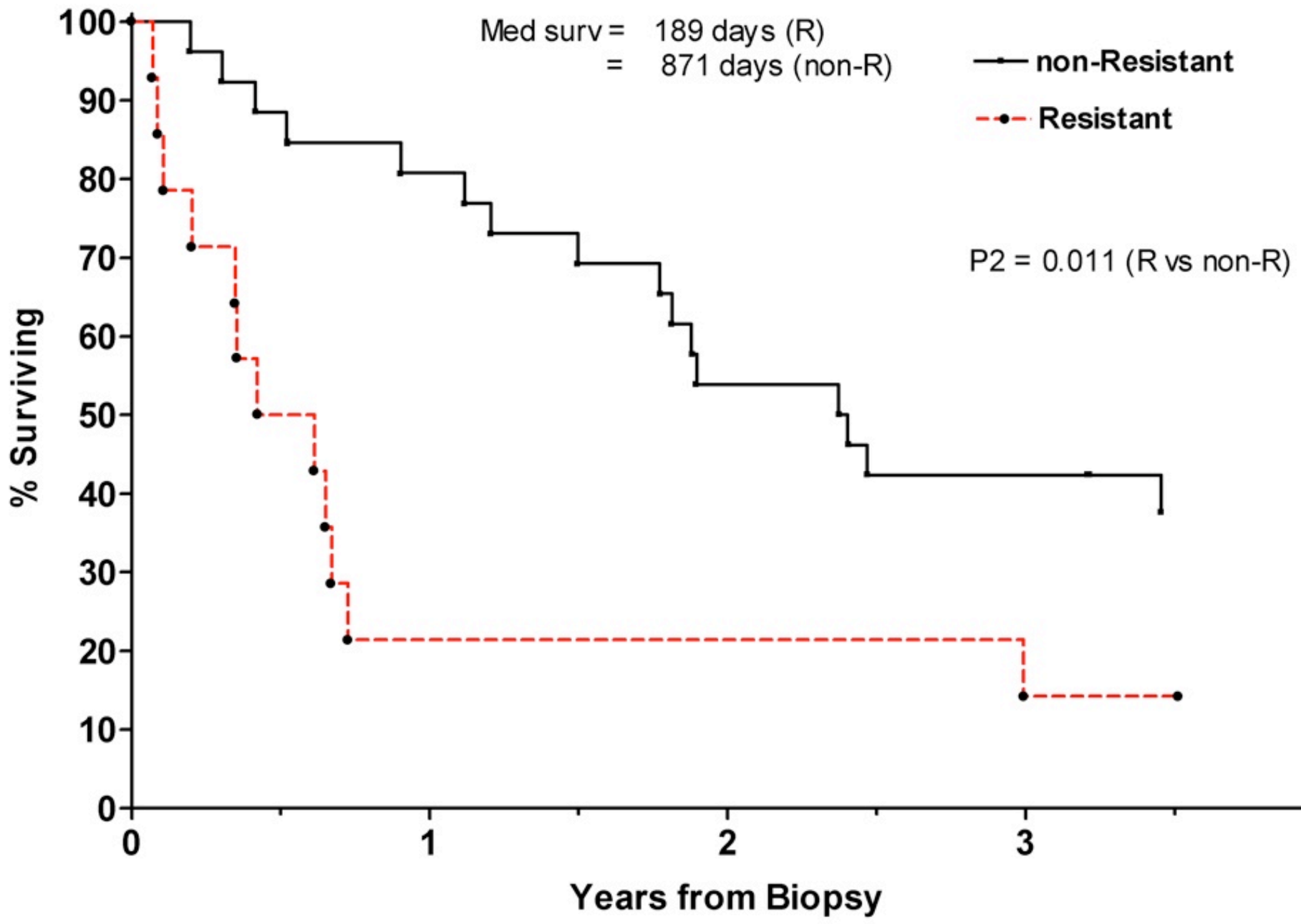
Stage IV Colon Cancer
Previously-Untreated
Survival as a function
of 5FU activity in vitro
(MTT Assay; 40 ug/ml; 96 hrs)
Stage 4



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



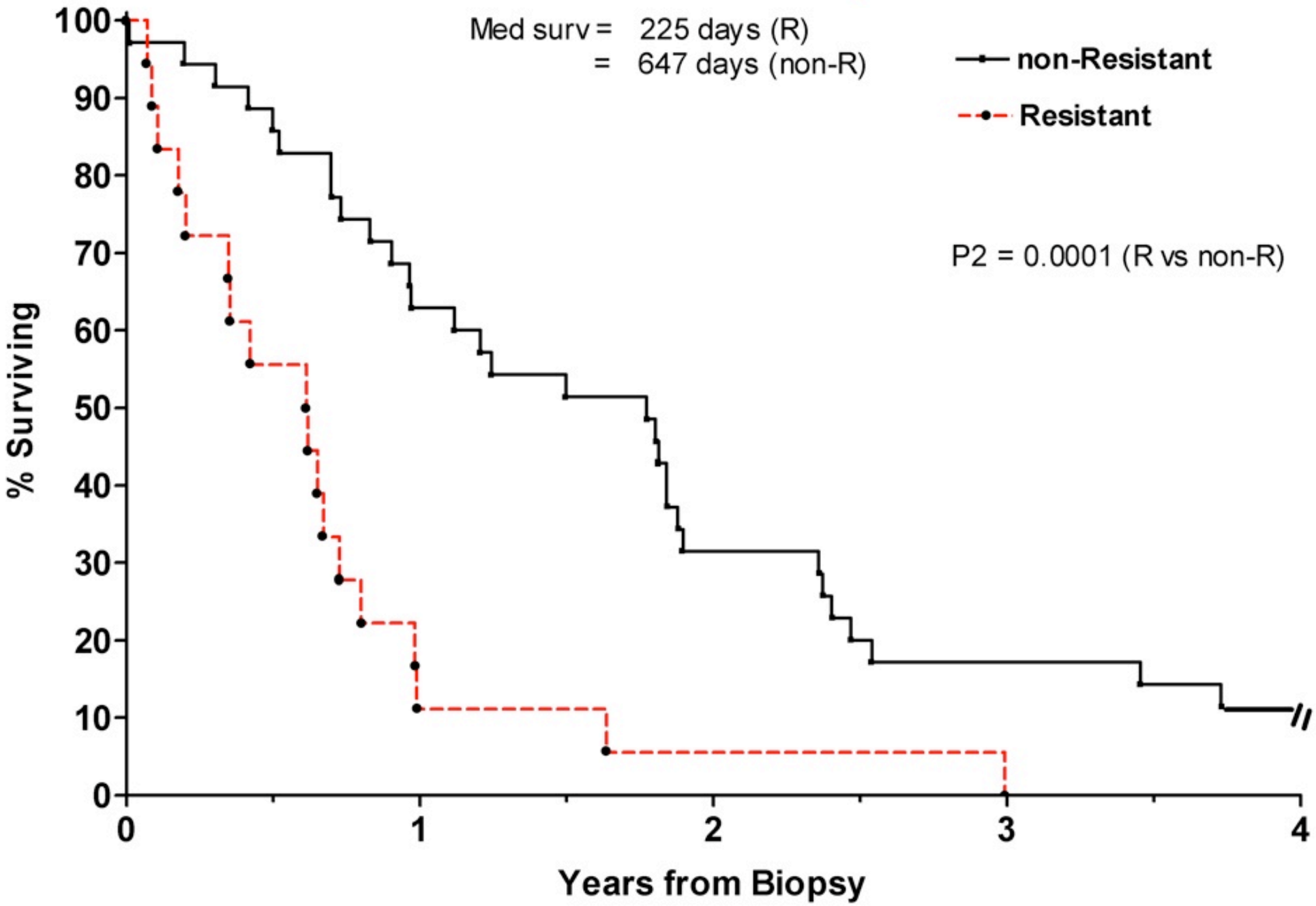
Stage IV Colon Cancer
Previously-Untreated
Survival as a function
of 5FU activity in vitro
(MTT Assay; 40 ug/ml; 96 hrs)
Stage 4; Both DISC/MTT Evaluable



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

Med surv = 225 days (R)
= 647 days (non-R)



Presentation outline

- Need for individualized therapy (efficacy,cost)
- Appropriate criteria for evaluating predictive tests: Accuracy vs. "Efficacy," example: Estrogen Receptor IHC, Oncotype Dx multigene expression test
- **Very brief and broad overview of data pertaining to cell culture assays**
- Detailed consideration of a single example: chronic lymphocytic leukemia
- Cell culture assays for "targeted" drugs
- Cell culture assay for anti-microvascular drugs

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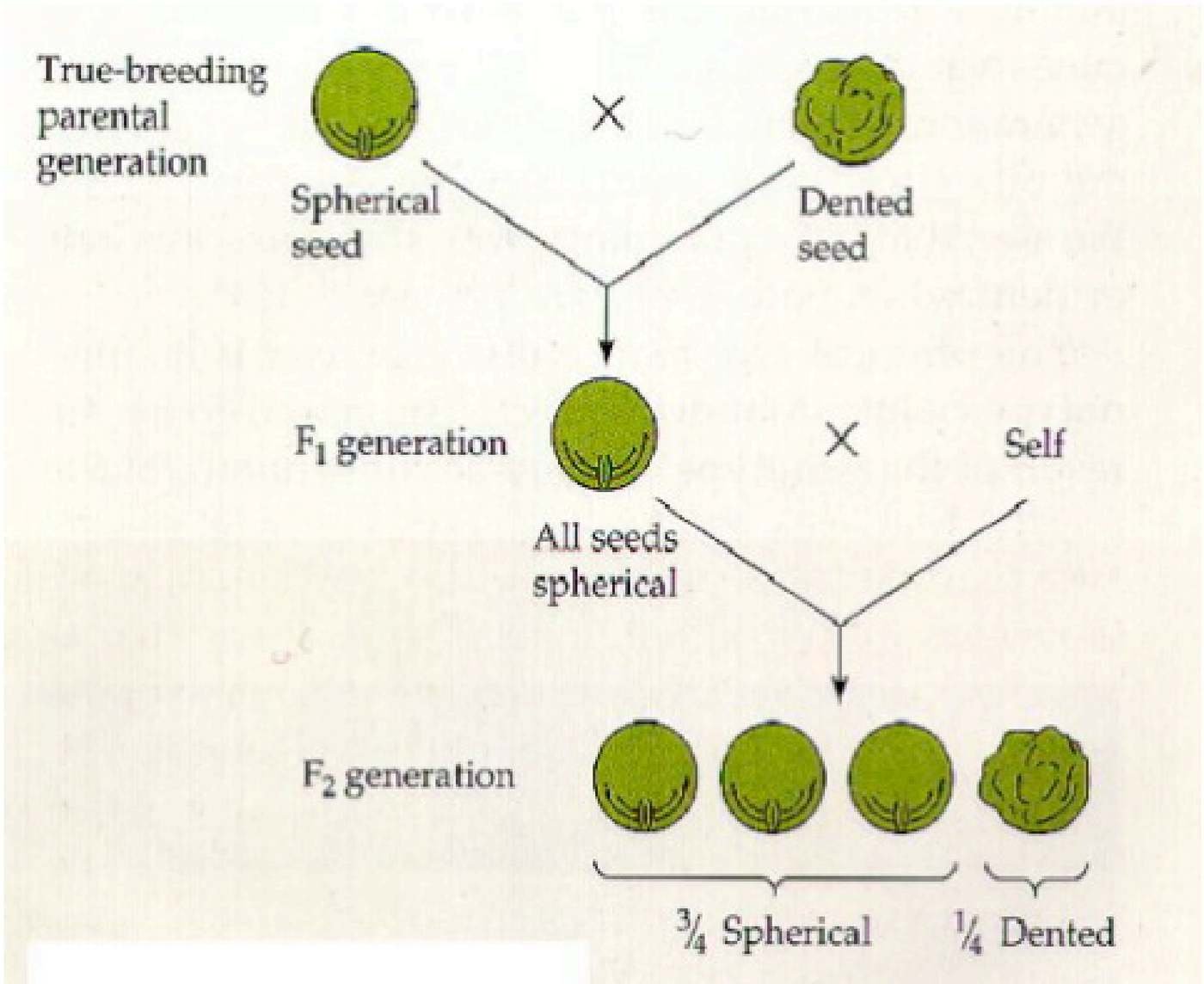
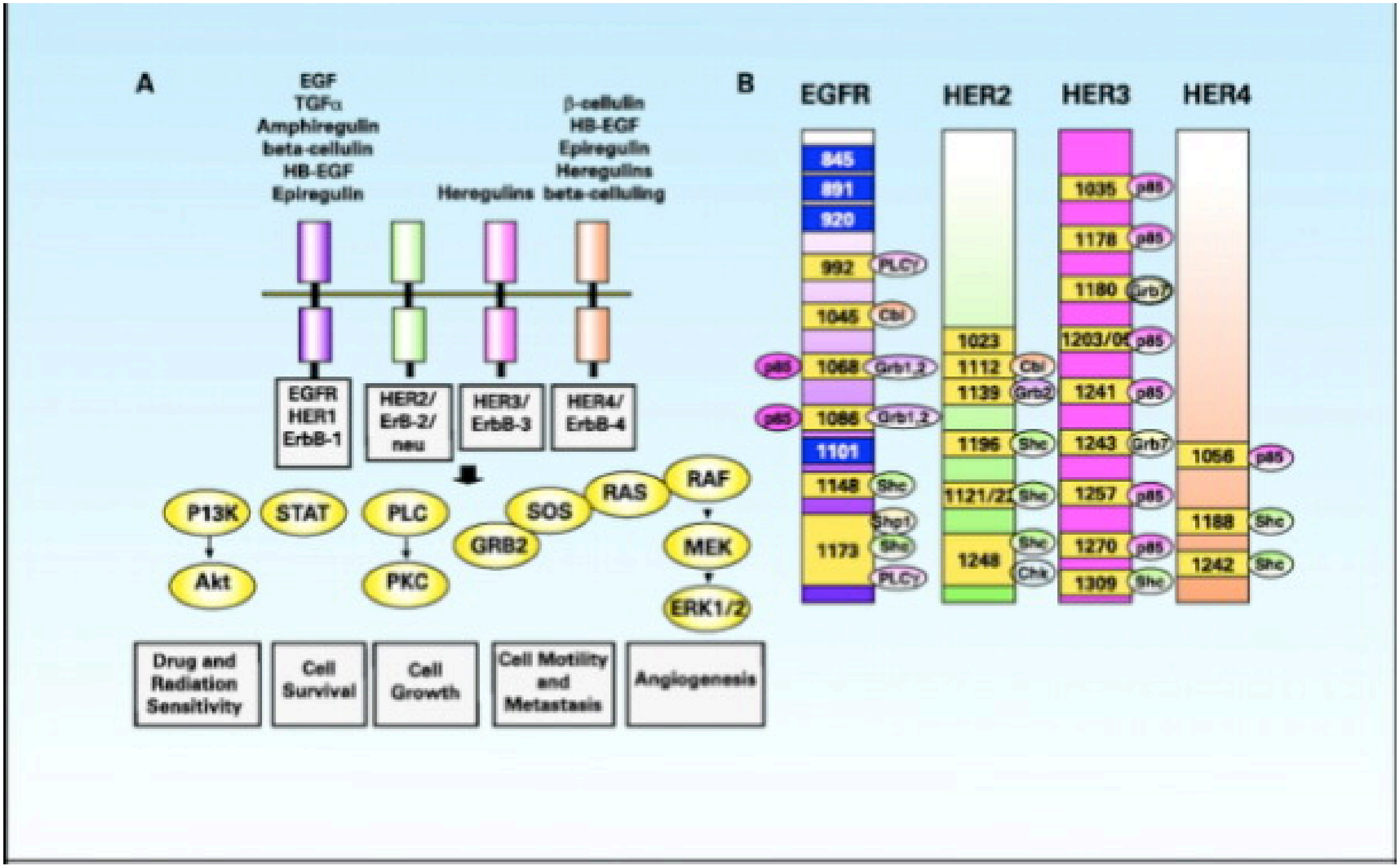
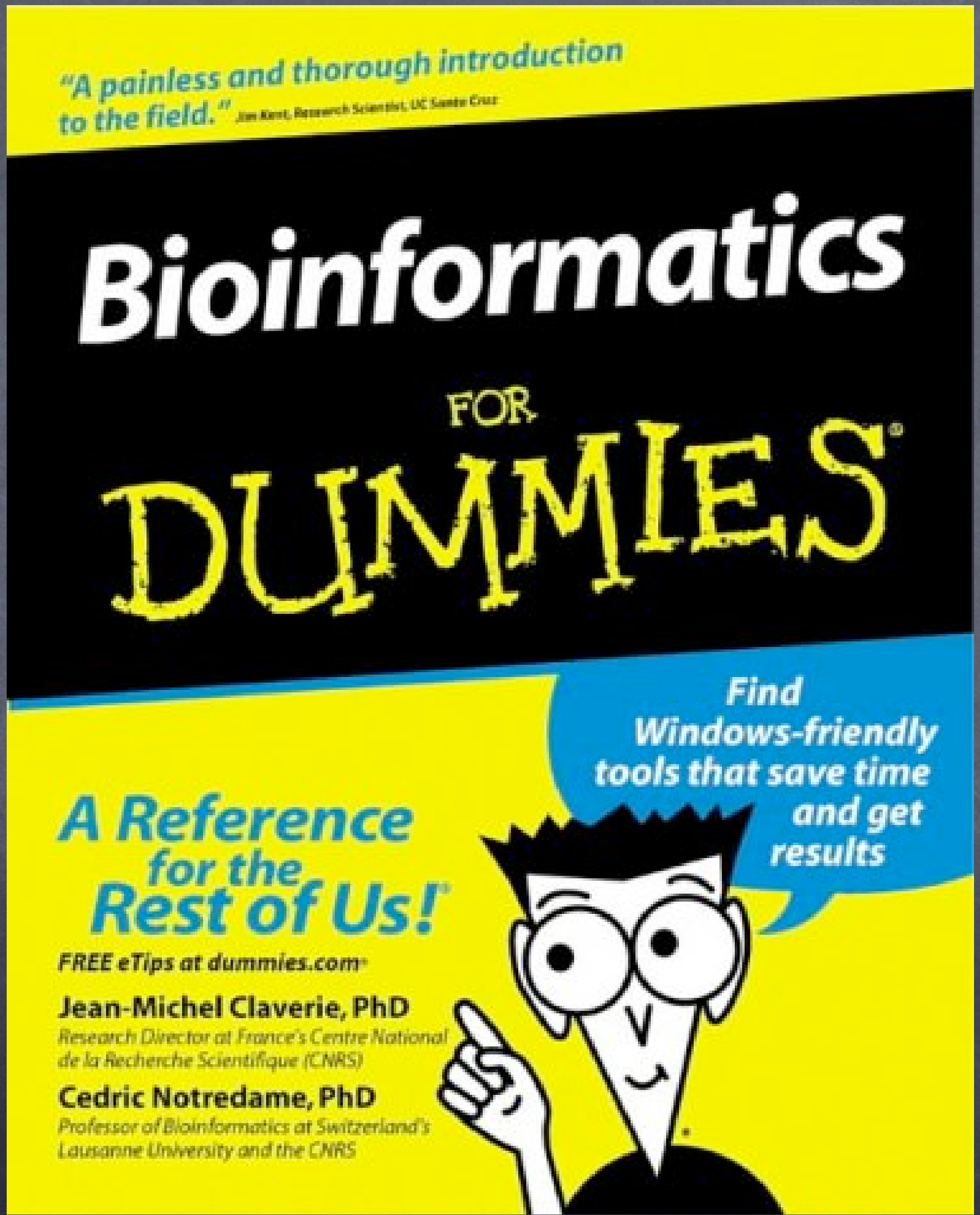


Mendel: Single Gene

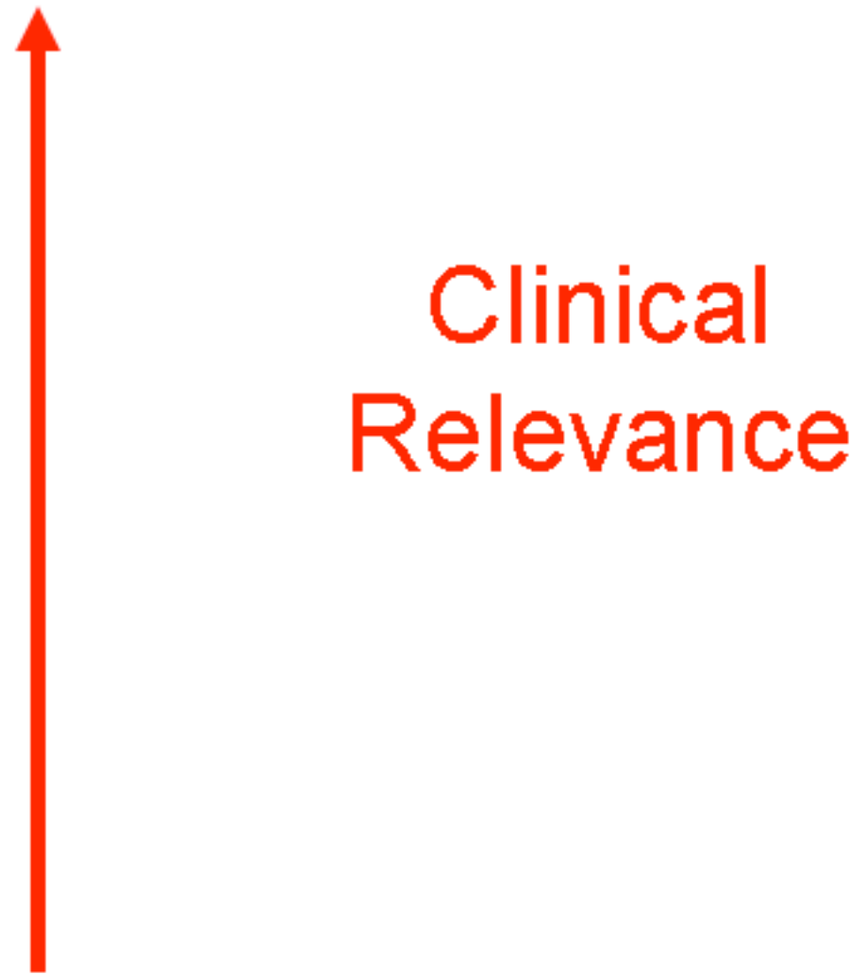


Multiple gene "target" for therapy: How would you test for synergy between bevacizumab + imatininib?


"A painless and thorough introduction to the field." Jim Kent, Research Scientist, UC Santa Cruz
Bioinformatics
FOR
DUMMIES
Find Windows-friendly tools that save time and get results
A Reference for the Rest of Us!
FREE eTips at dummies.com
Jean-Michel Claverie, PhD
Research Director at France's Centre National de la Recherche Scientifique (CNRS)
Cedric Notredame, PhD
Professor of Bioinformatics at Switzerland's Lausanne University and the CNRS

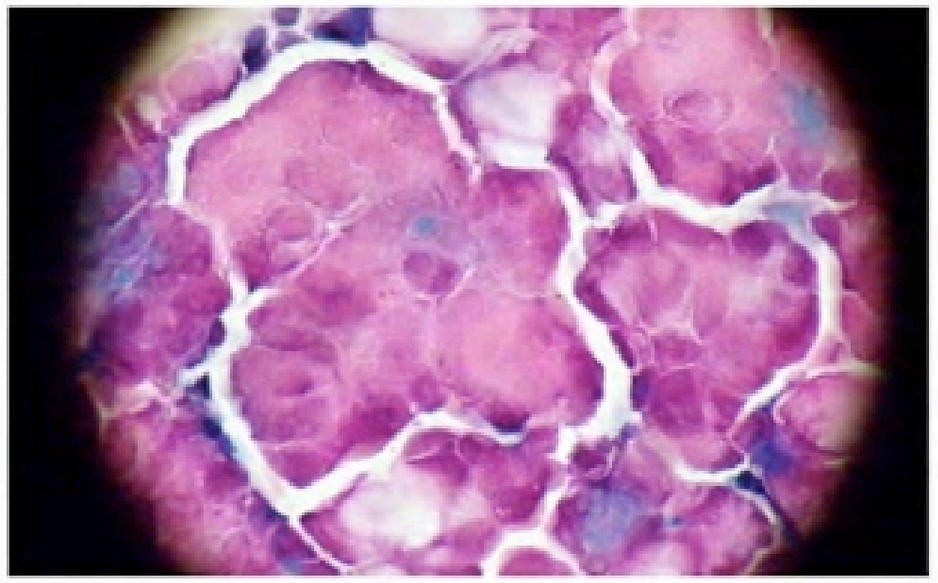
What is the *best* endpoint?

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



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			



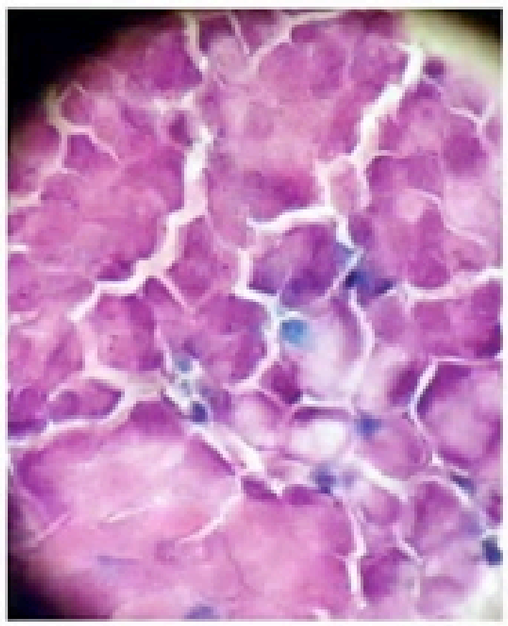
Control Culture

EGFRx™ Assay Detail

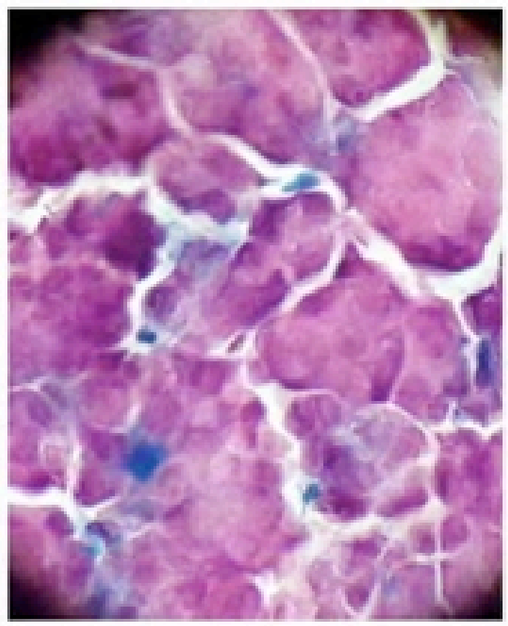
Assay/Analysis Name:	EGFRx
Analysis Type:	Whole Cell Profiling
Endpoint:	Cell morphology plus cell metabolism
Agent Class:	Kinase Inhibitor
Pathway/Mechanism:	EGFR/ Kinase Signaling

EGFRx™ Assay - Cellular Response Profiles

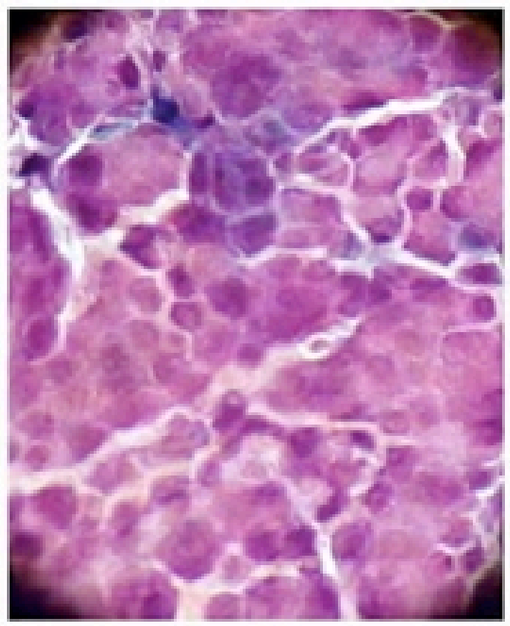
Targeted Therapy Agent	Drug Activity	Activity Catagory
Erlotinib (Tarceva)	Low	Unfavorable
Gefitinib (Iressa)	Low	Unfavorable
Sunitinib (Sutent)	Low	Unfavorable



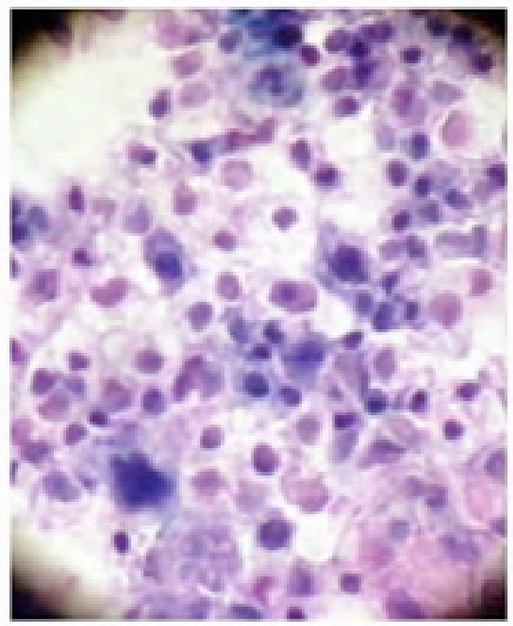
Erlotinib



Gefitinib



Sunitinib

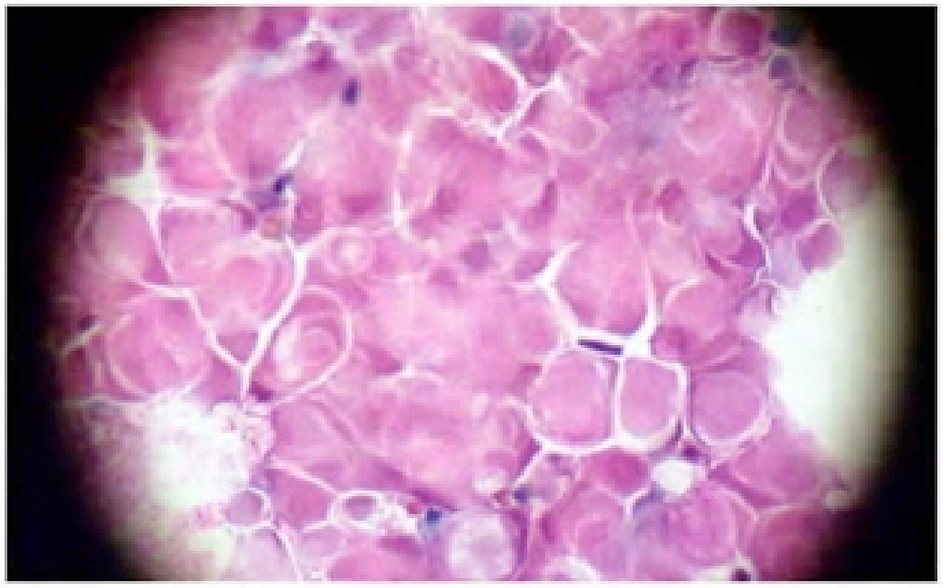


Sorafenib

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



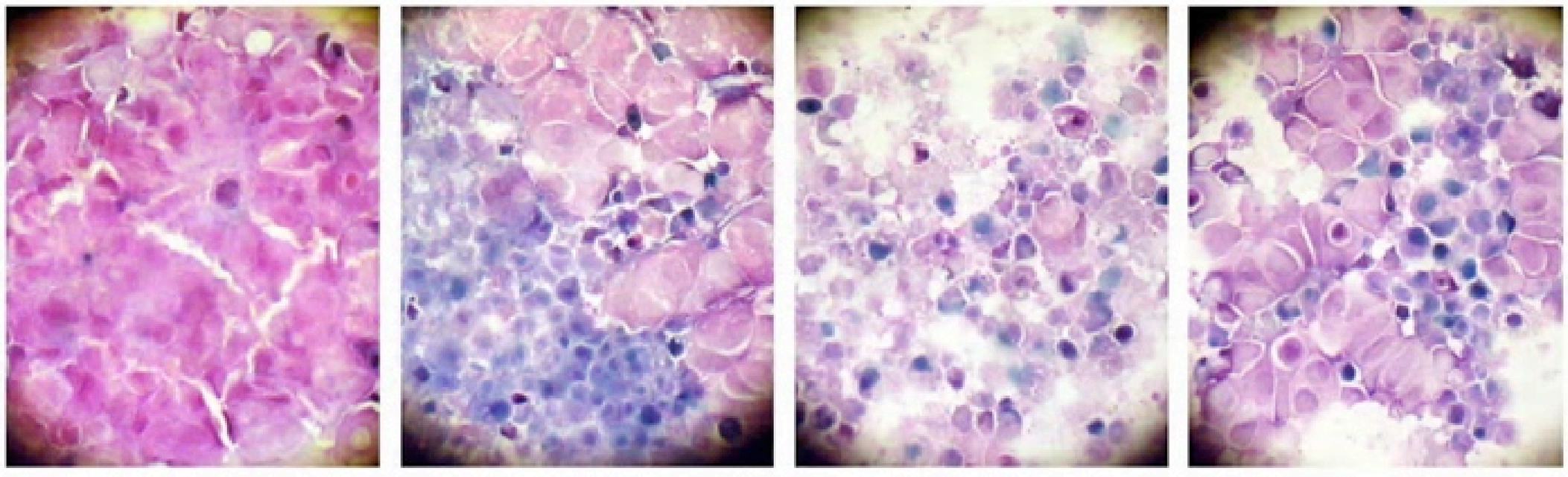
Control Culture

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 Catagory
Erlotinib (Tarceva)	Low	Unfavorable
Gefitinib (Iressa)	Moderate	Borderline
Sorafenib	Moderate	Borderline
Sunitinib (Sutent)	High	Favorable

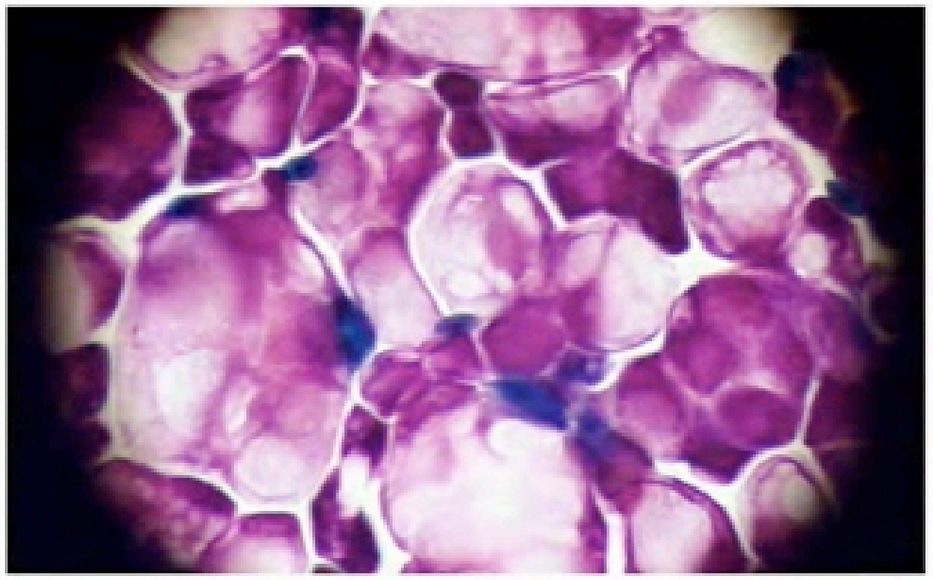


Erlotinib Gefitinib Sunitinib Sorafenib

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.



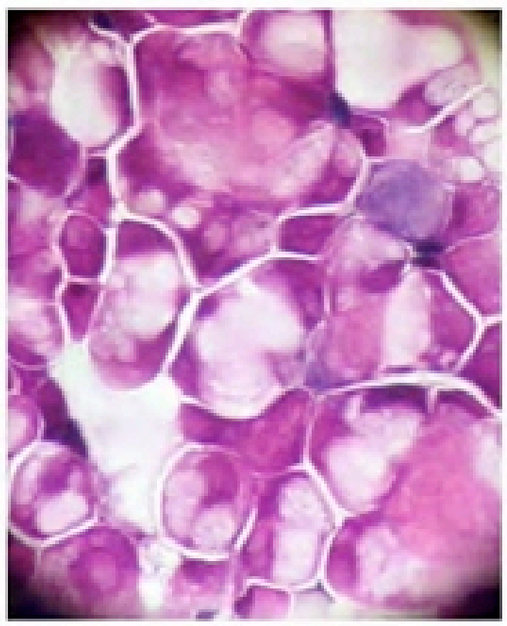
Control Culture

EGFRx™ Assay Detail

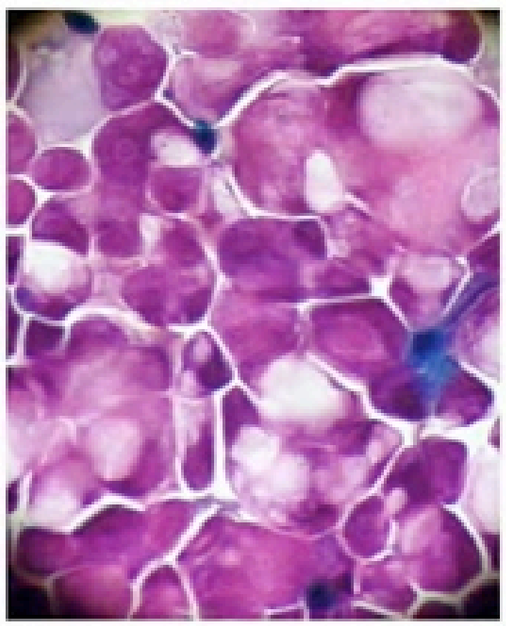
Assay/Analysis Name:	EGFRx
Analysis Type:	Functional Cell Profiling
Endpoint:	Cell Metabolism/Cell Morphology
Agent Class:	Kinase Inhibitors
Pathway/Mechanism:	EGFR/Kinase Signaling

EGFRx™ Assay - Cellular Response Profiles

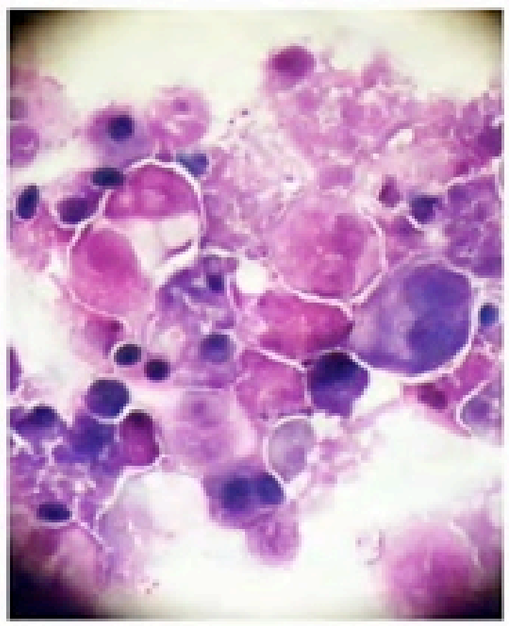
Targeted Therapy Agent	Drug Activity	Activity Catagory
Erlotinib (Tarceva)	Low	Unfavorable
Gefitinib (Iressa)	Low	Unfavorable
Sunitinib (Sutent)	Moderate	Borderline
Sorafenib (Nexavar)	High	Favorable



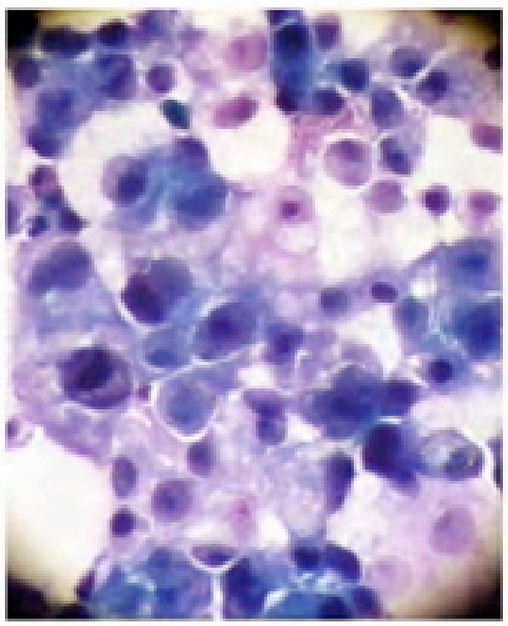
Erlotinib



Gefitinib



Sunitinib

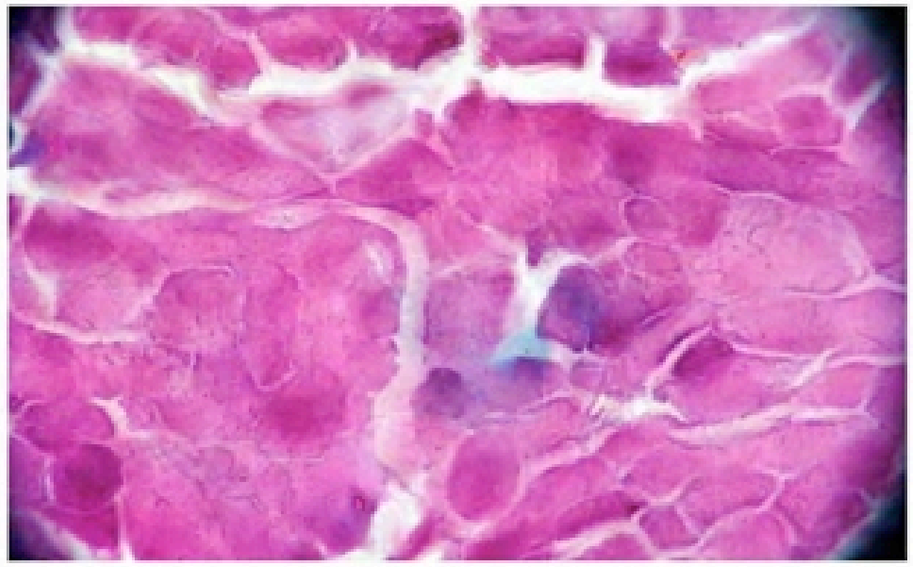


Sorafenib

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.



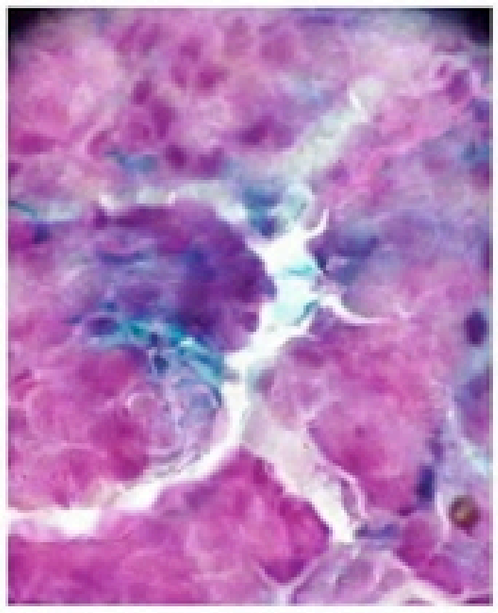
Control Culture

EGFRx ™ Assay Detail

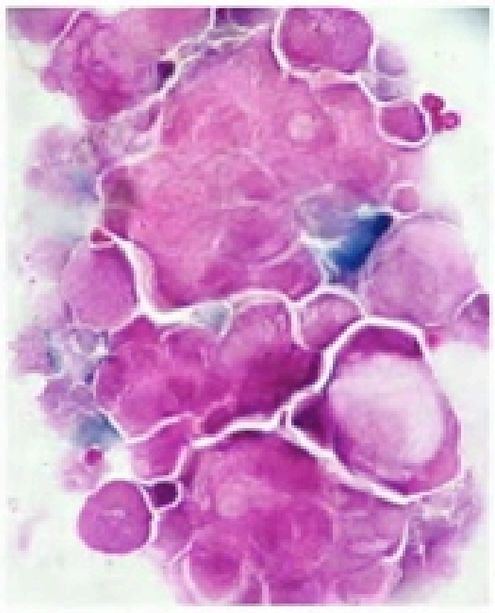
Assay/Analysis Name:	EGFRx
Analysis Type:	Functional Profiling
Endpoint:	Cell Metabolism + Cell Morphology
Agent Class:	Kinase Inhibitor
Pathway/Mechanism:	EGFR/Kinase Signaling

EGFRx ™ Assay - Cellular Response Profiles

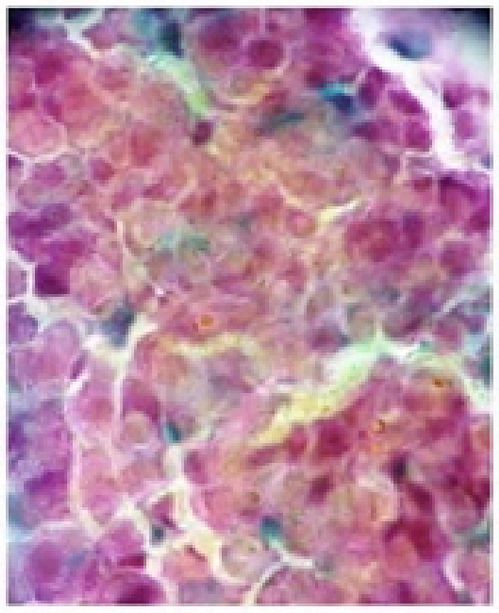
Targeted Therapy Agent	Drug Activity	Activity Catagory
Erlotinib (Tarceva)	Low	Unfavorable
Gefitinib (Iressa)	Low	Unfavorable
Sorafenib	Low	Unfavorable
Sunitinib (Sutent)	Low	Unfavorable



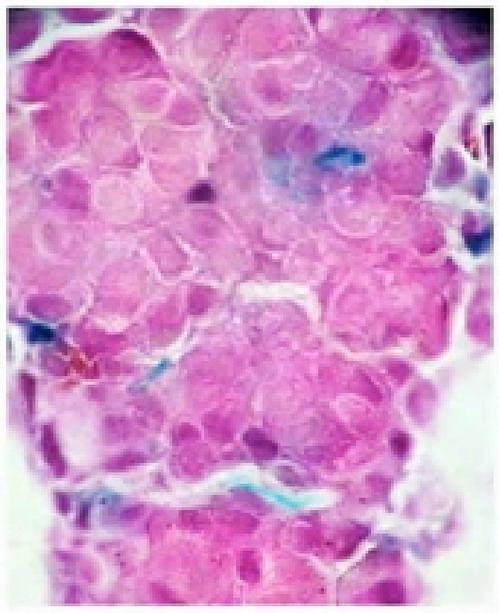
Erlotinib



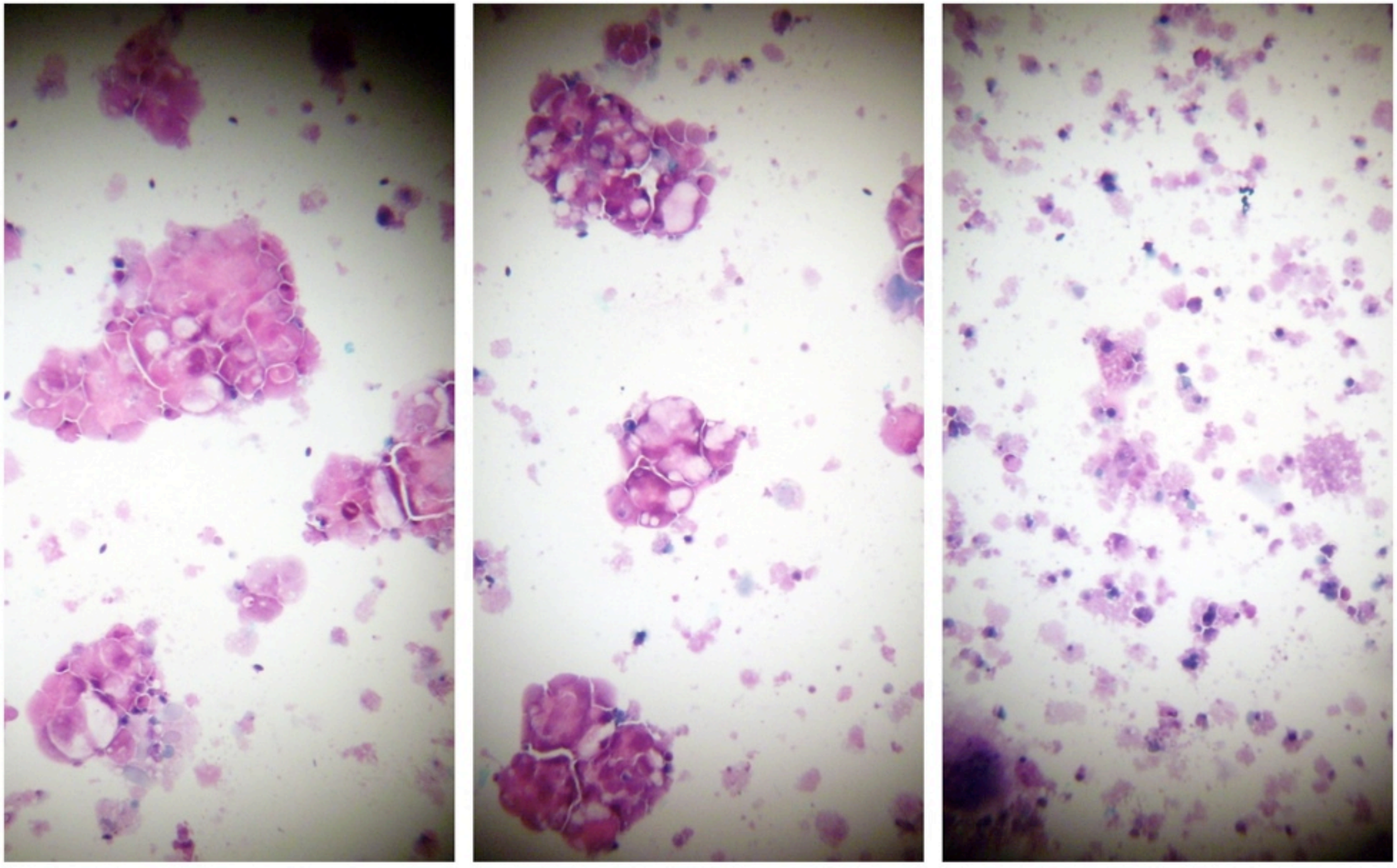
Gefitinib



Sunitinib



Sorafenib

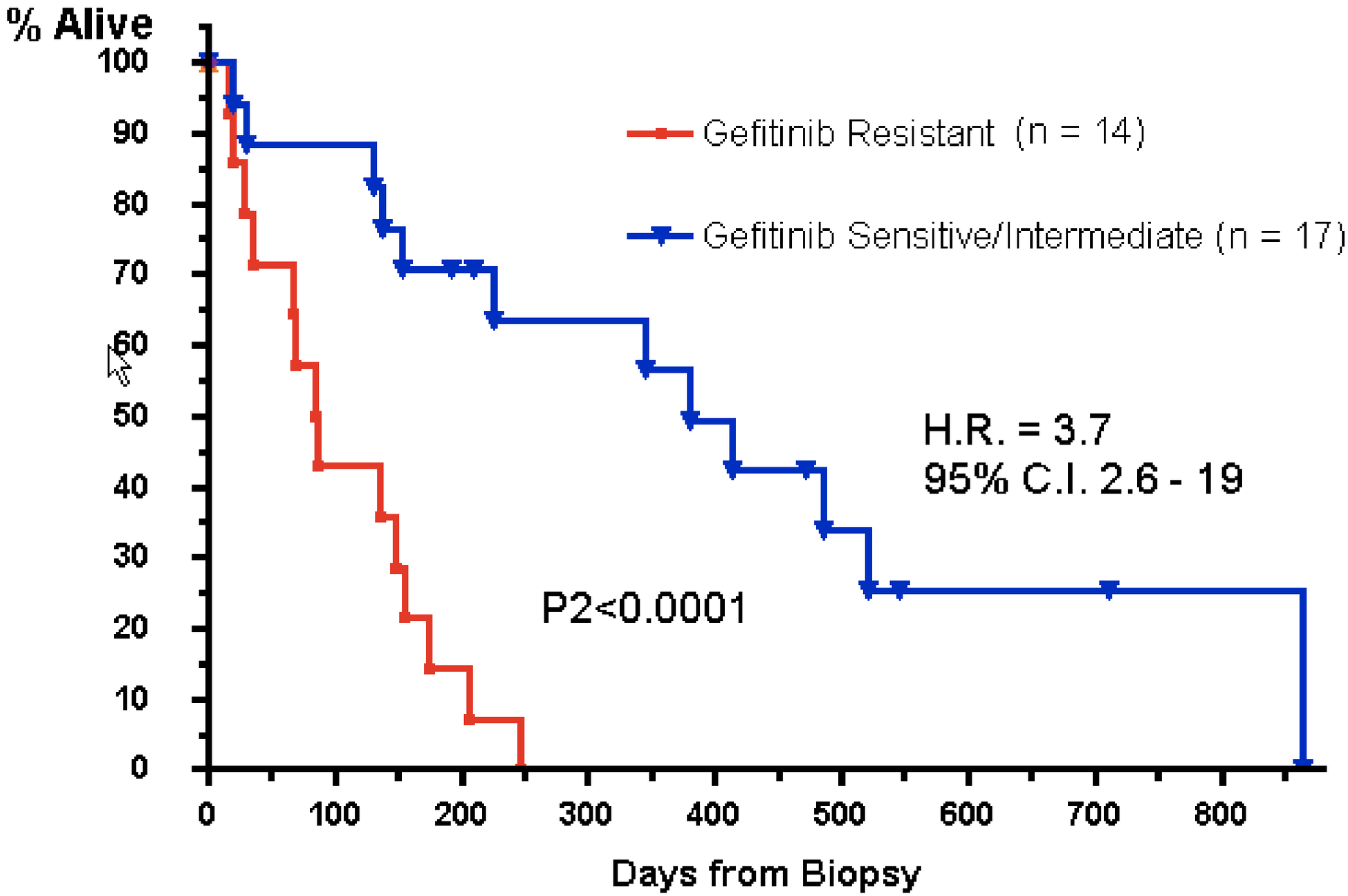


Ovarian
VNRLB 10

Ovarian
GEFIT 11

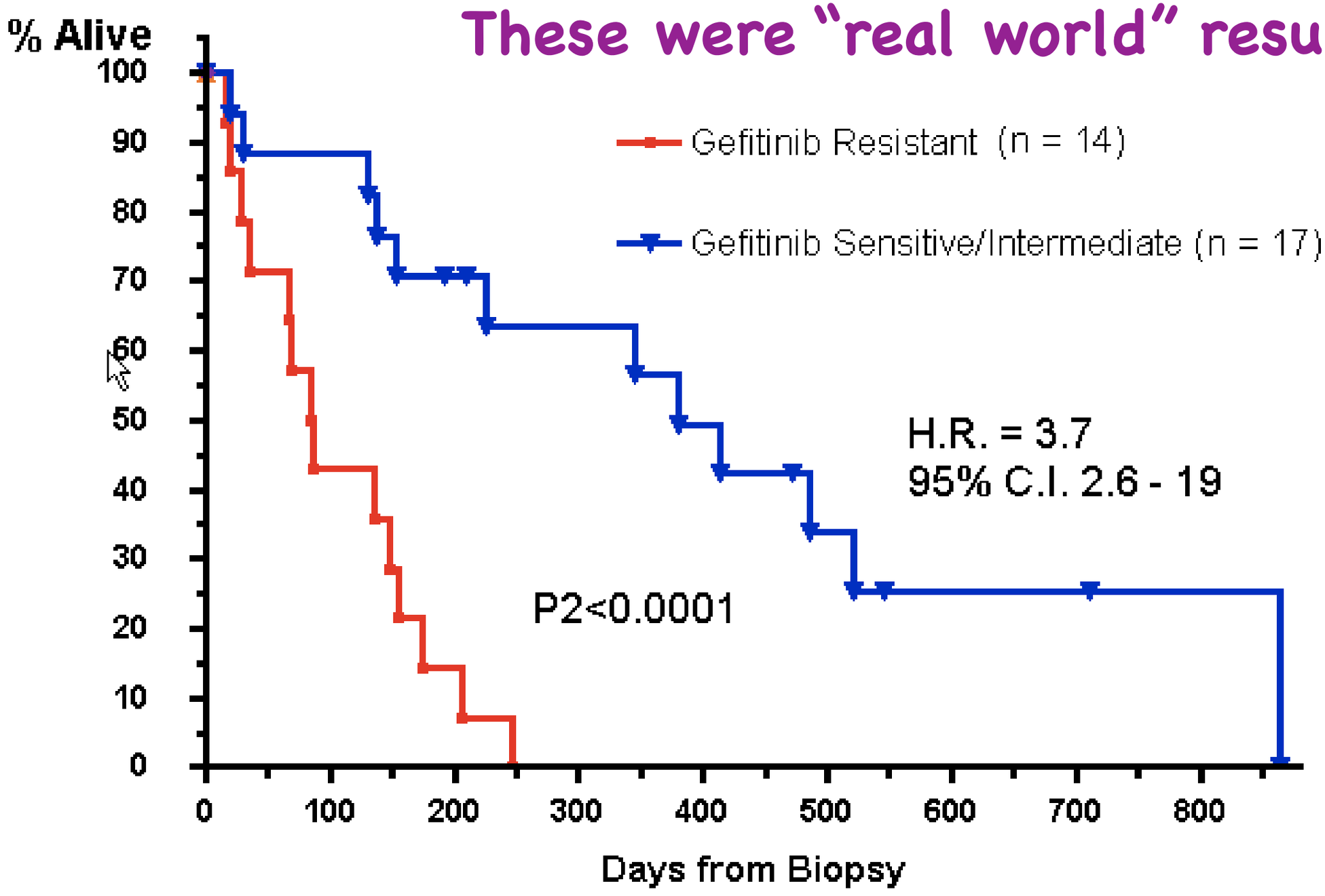
Ovarian
VNRLB 5 +
Gefit 5.5

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

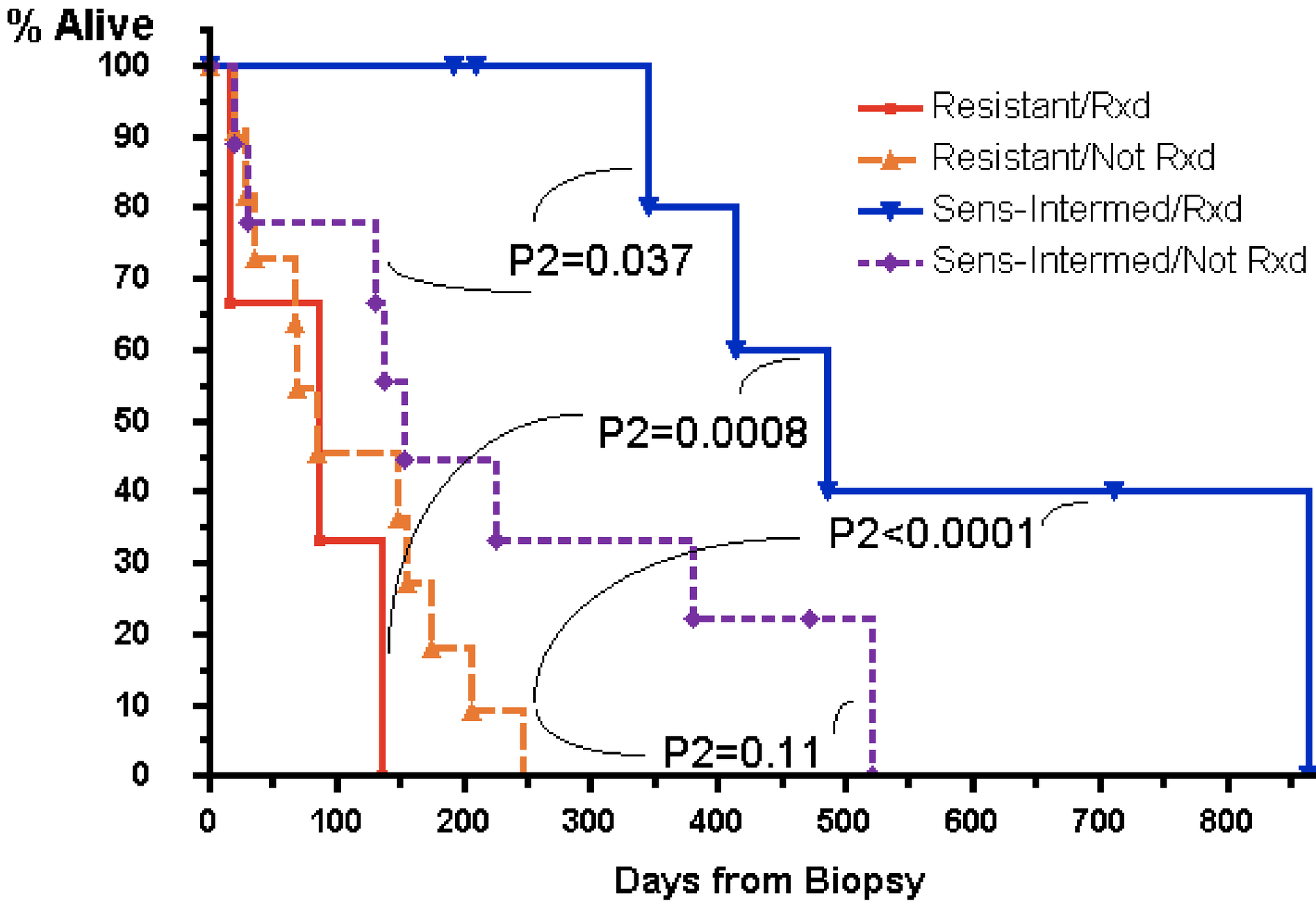


Previously-Treated NSCLC, Survival as Function of Cell Death Assay Results (as Reported Prospectively)

These were "real world" results



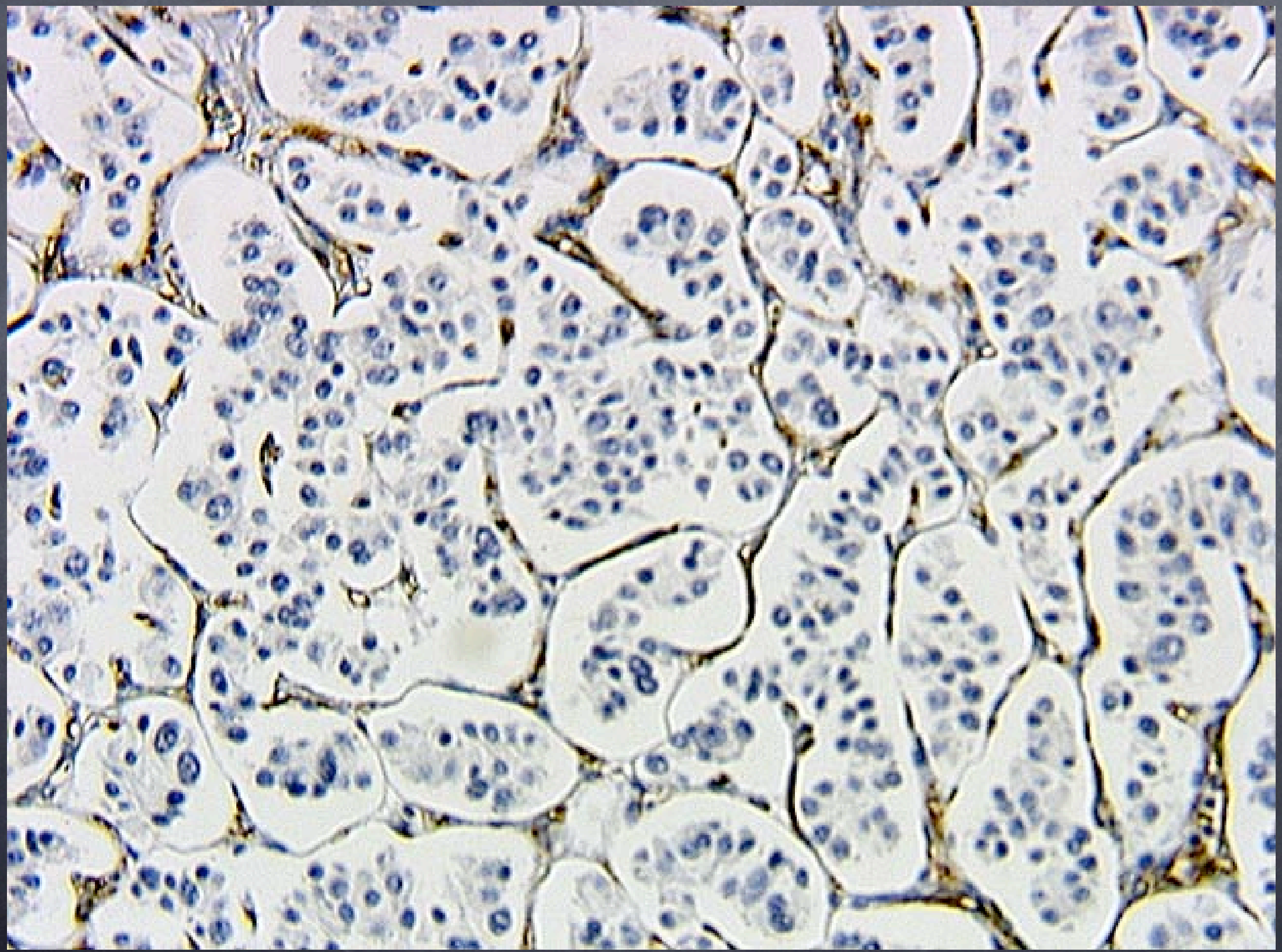
Previously-Treated NSCLC, Survival as Function of Assay Results (as Reported Prospectively) and Gefitinib/Erlotinib Treatment



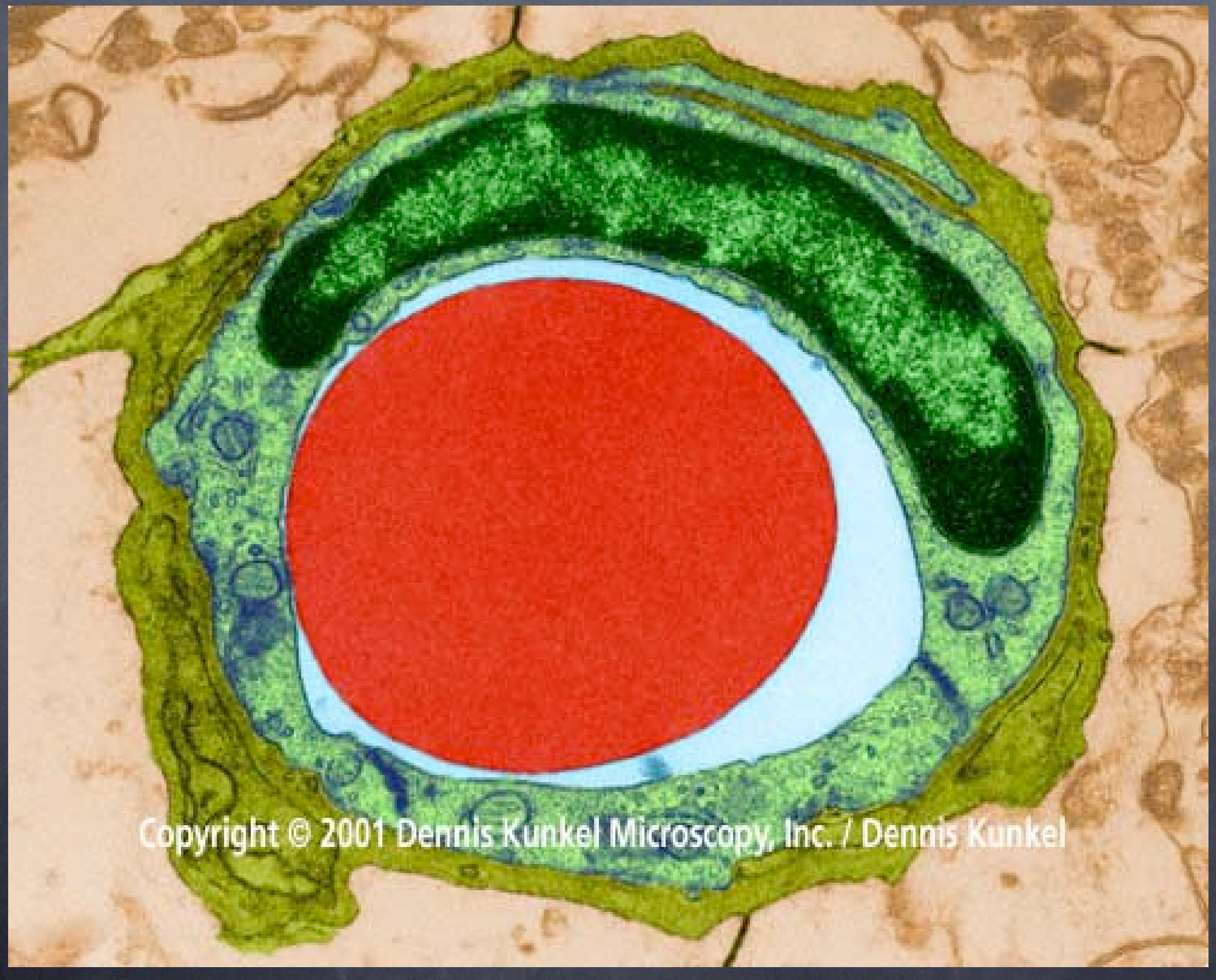
Presentation outline

- Need for individualized therapy (efficacy,cost)
- Appropriate criteria for evaluating predictive tests: Accuracy vs. "Efficacy," example: Estrogen Receptor IHC, Oncotype Dx multigene expression test
- Very brief and broad overview of data pertaining to cell culture assays
- Detailed consideration of a single example: chronic lymphocytic leukemia
- Cell culture assays for "targeted" drugs
- **Cell culture assay for anti-microvascular drugs**

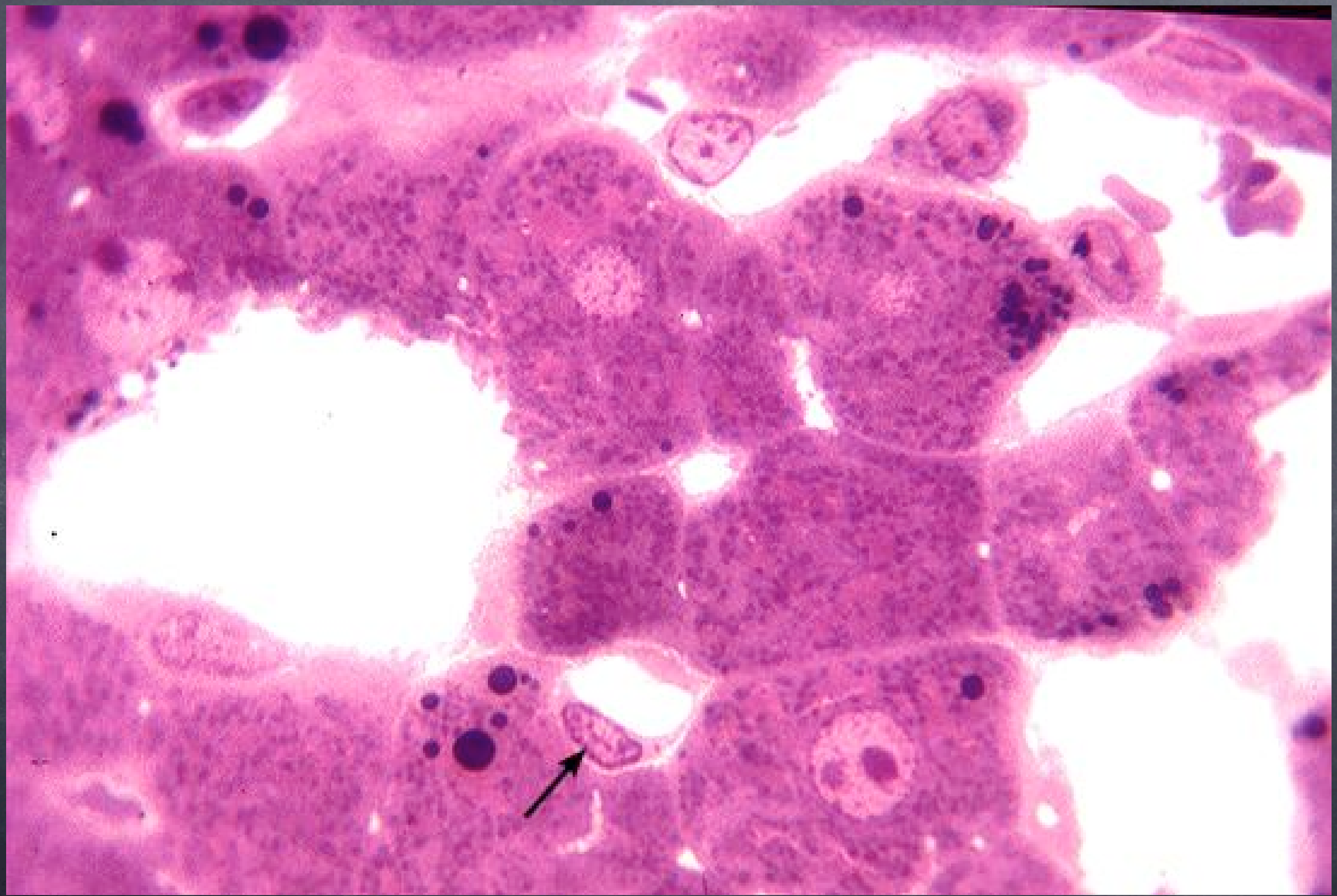
CD31 Stain in pancreatic Ca



Electron micrograph of single endothelial cell forming capillary



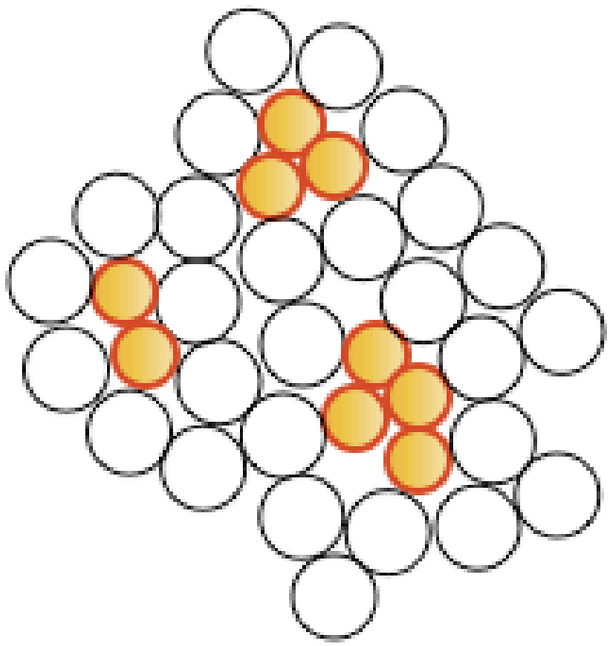
Endothelial cell forming capillary in tumor



Method for testing Anti-angiogenesis Drug In Vitro

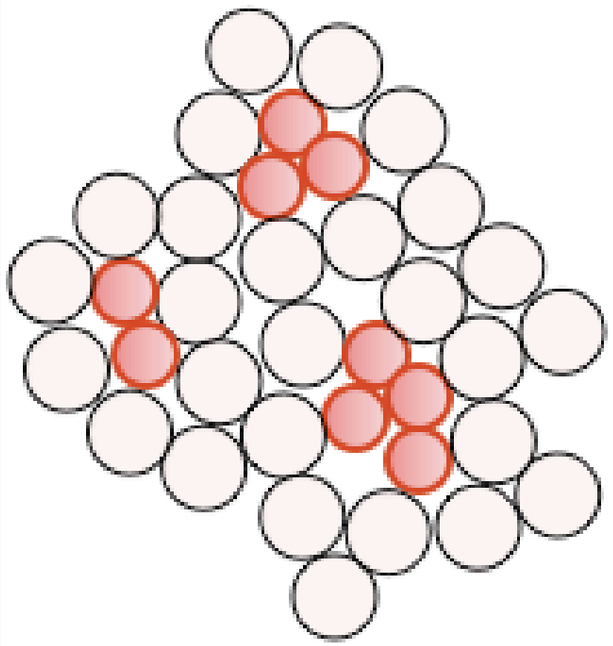
(© Weisenthal Cancer Group, patent pending)

Cells in Microcluster



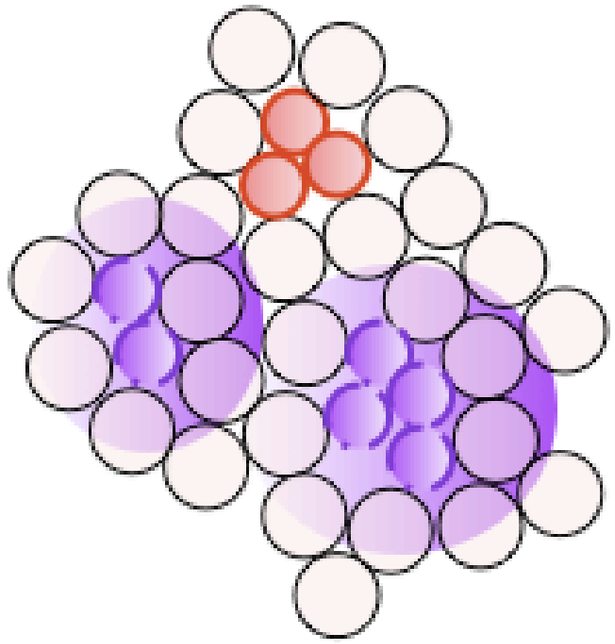
CD31 cytoplasmic staining confirms morphological identification of microcapillary cells in tumor microcluster

Negative Control



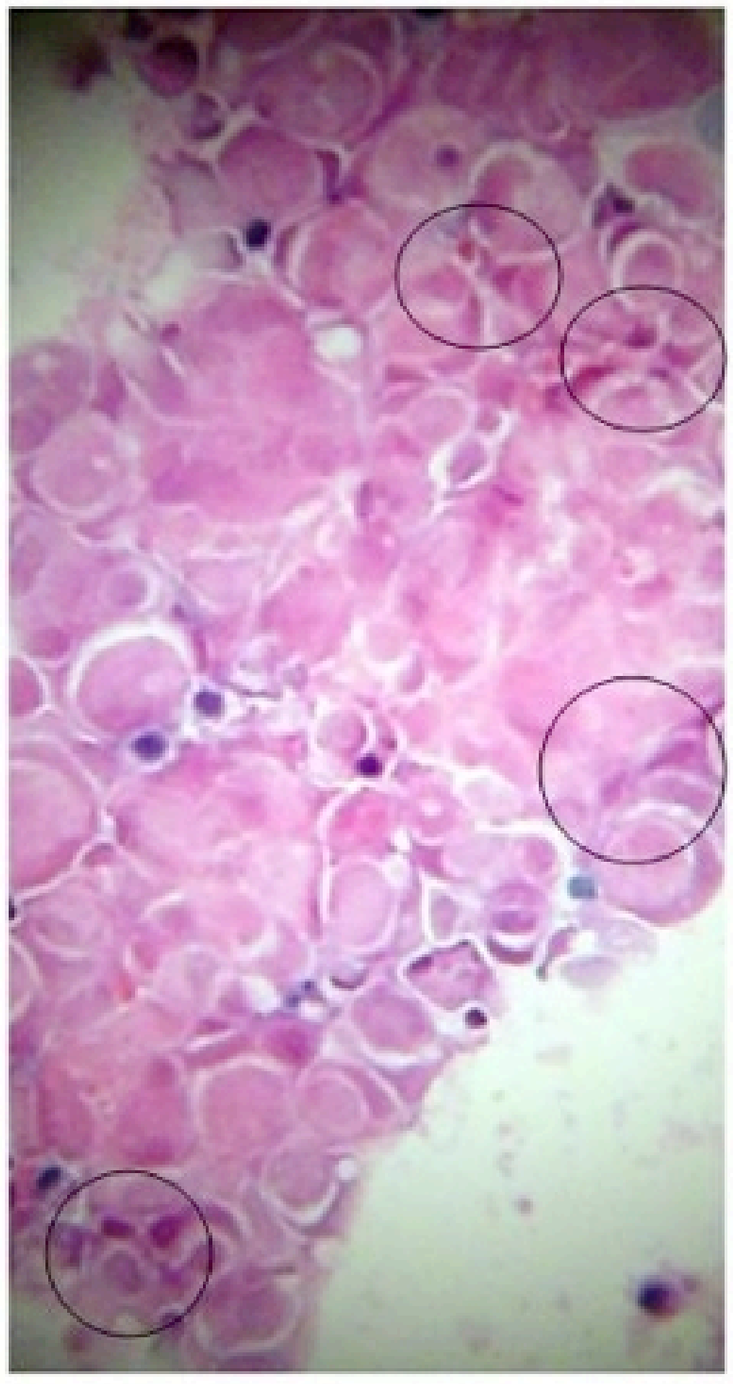
Living cells in culture, NOT exposed to anti-VEGF drug. Intact membranes of undamaged microcapillary cells exclude vital dye - no visible staining.

Drug Exposure (bevacizumab)

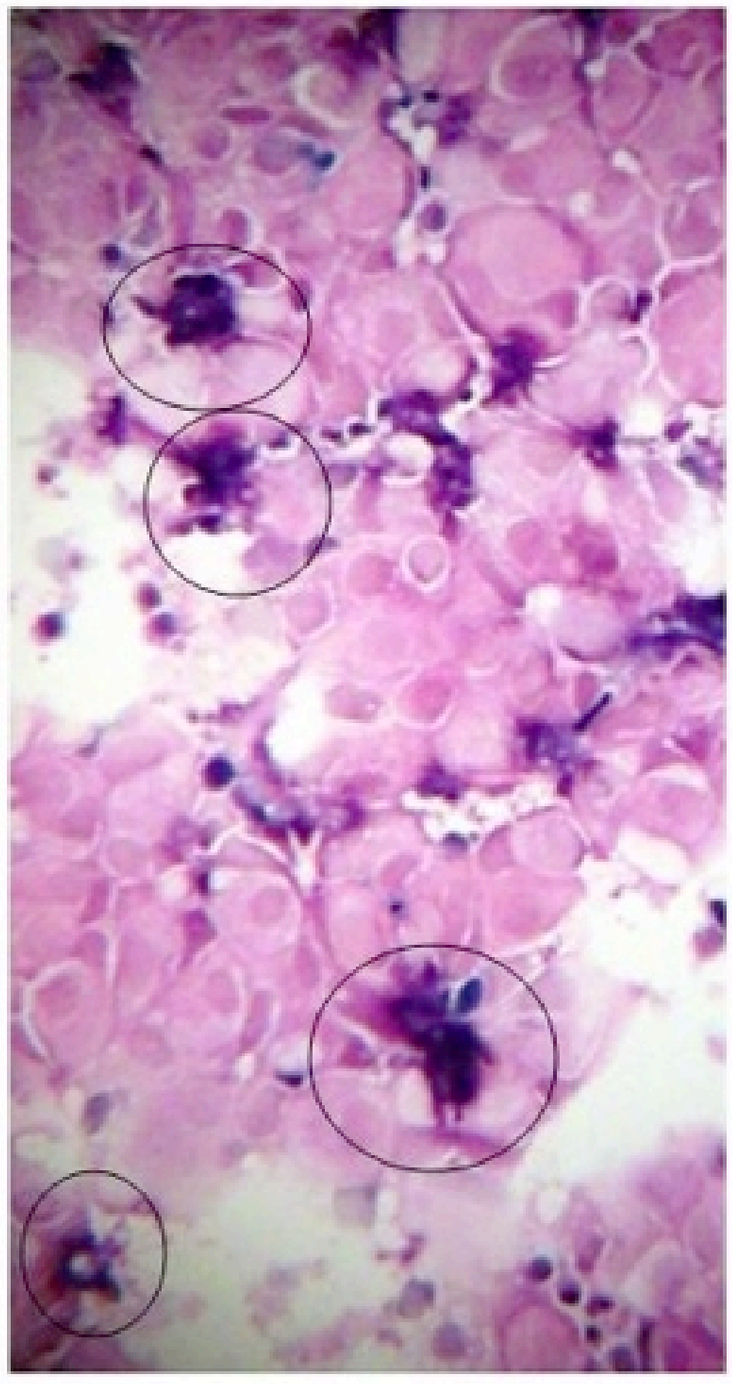


Leaky membranes of dead/ dying micro-capillary cells admit vital dye which then extrudes into adjacent spaces during alcohol-based counterstaining. Tumor cells are not harmed by anti-VEGF drug.

CD31

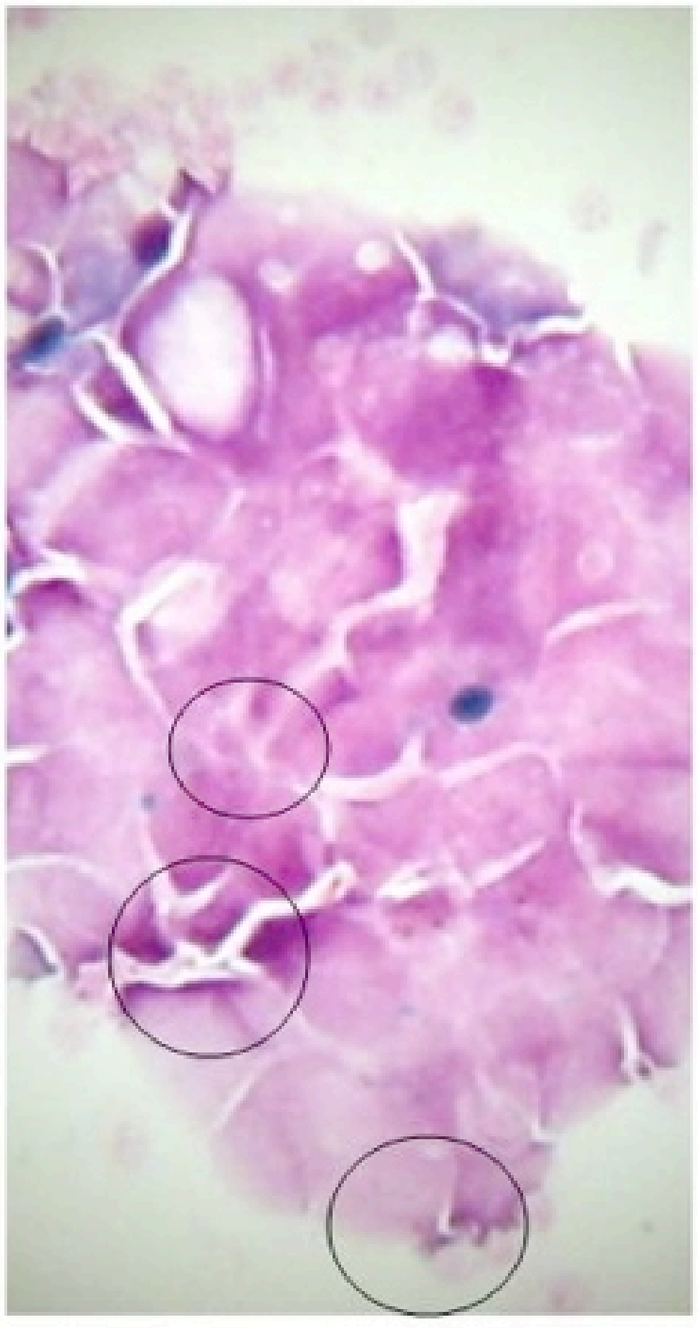


Control Culture



Bevacizu-mab

CD31



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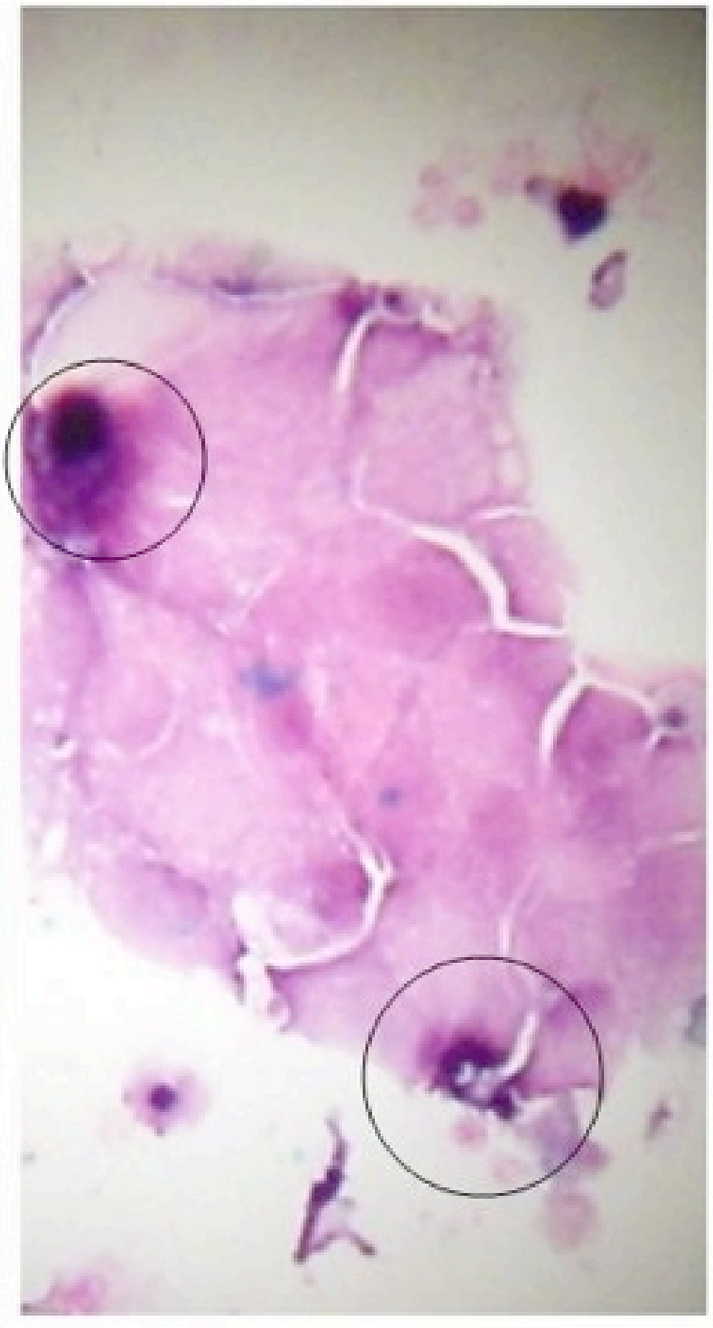
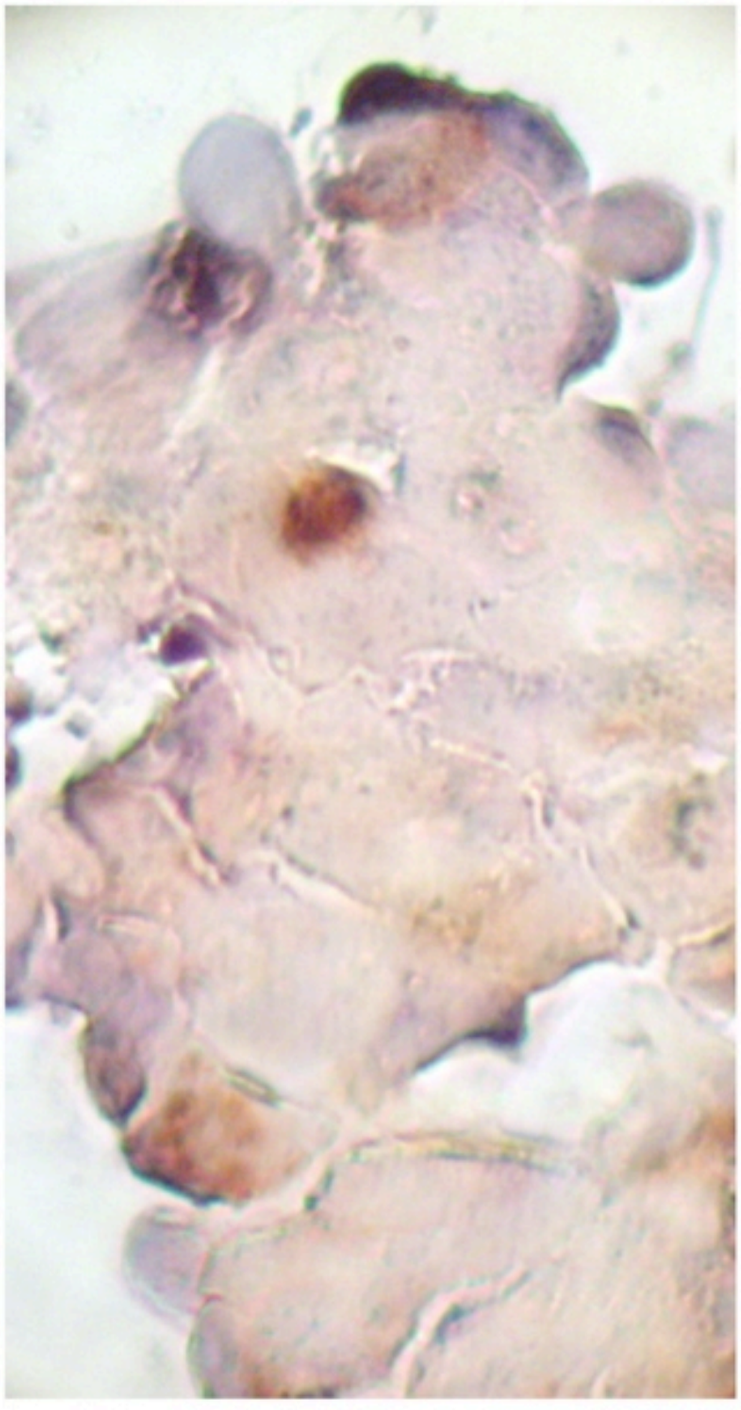


Bevacizu-mab



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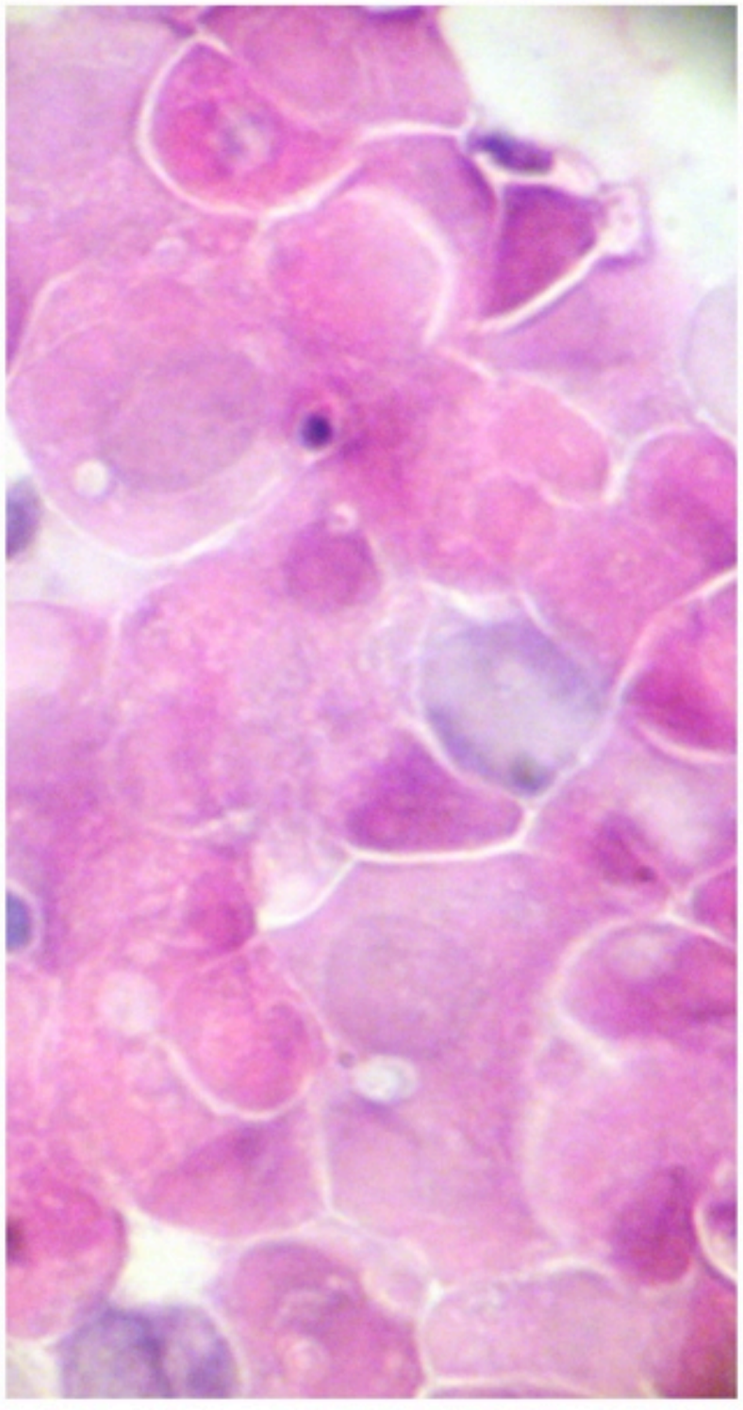
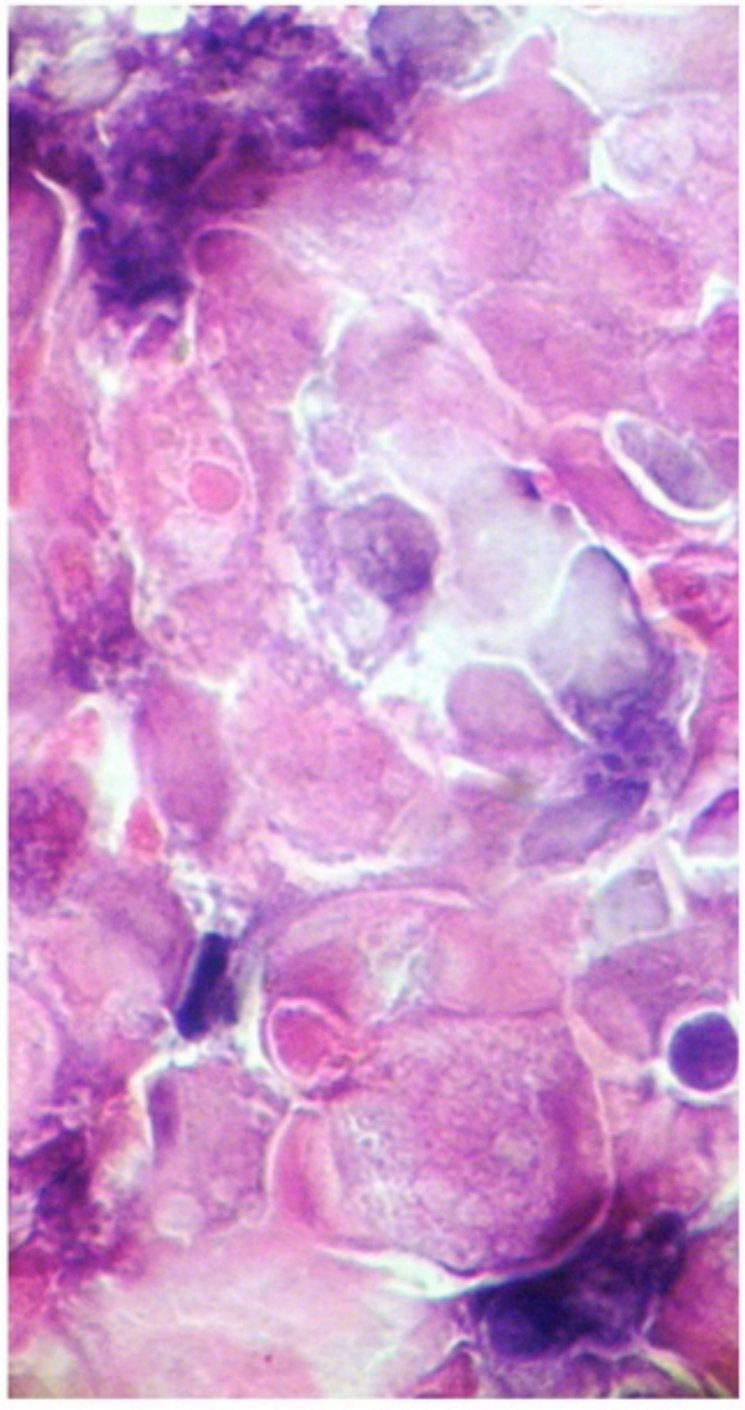


Control Culture



Bevacizu-mab

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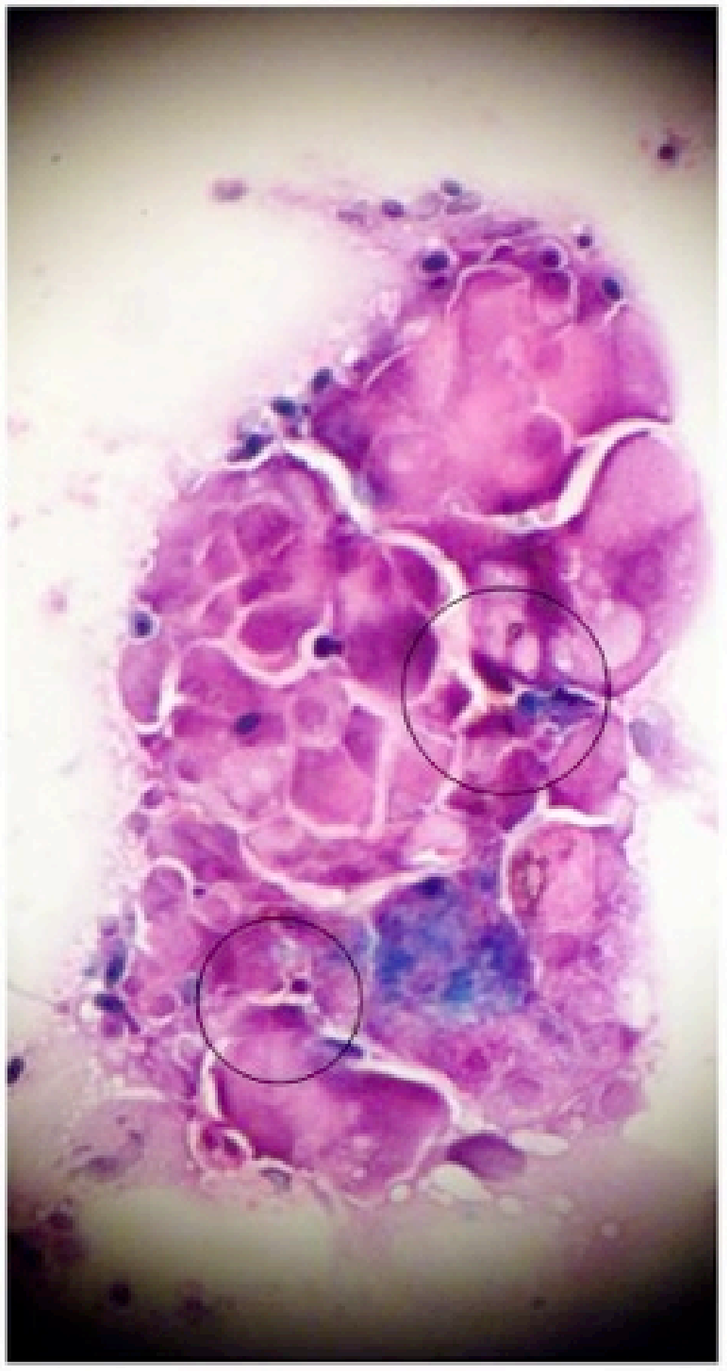
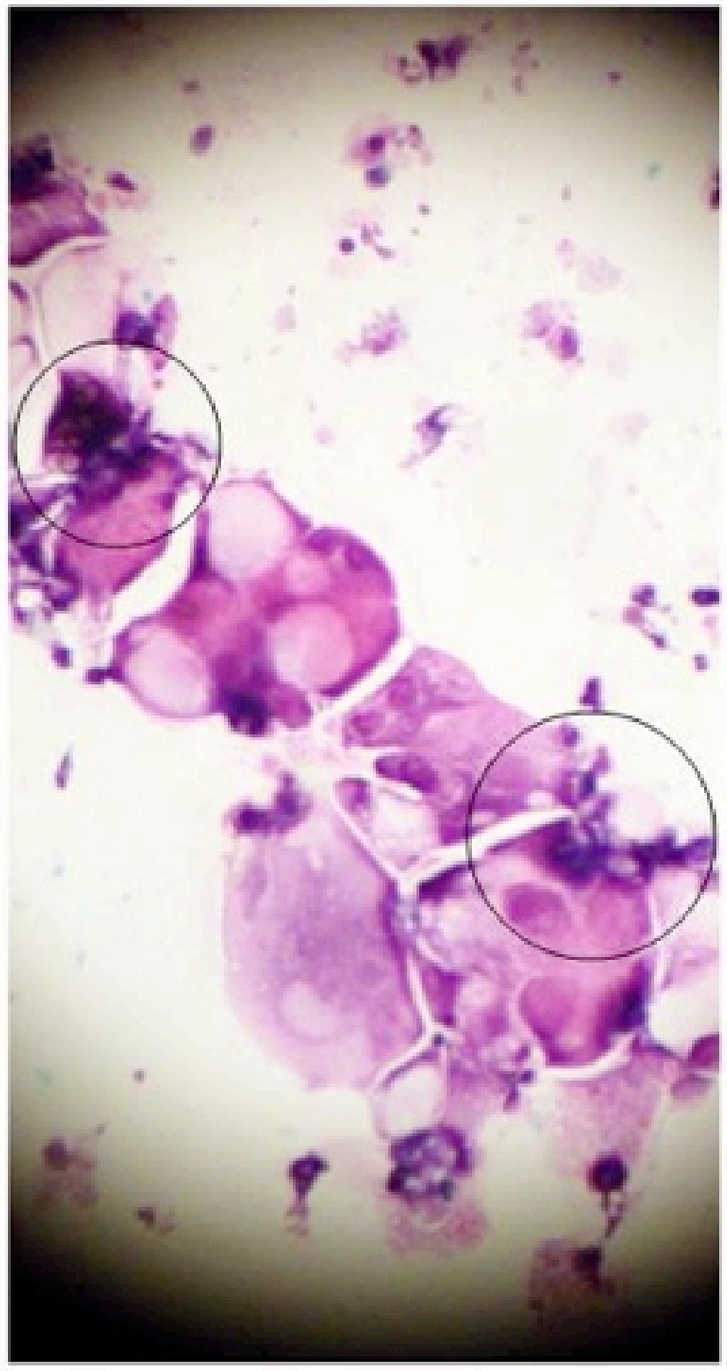
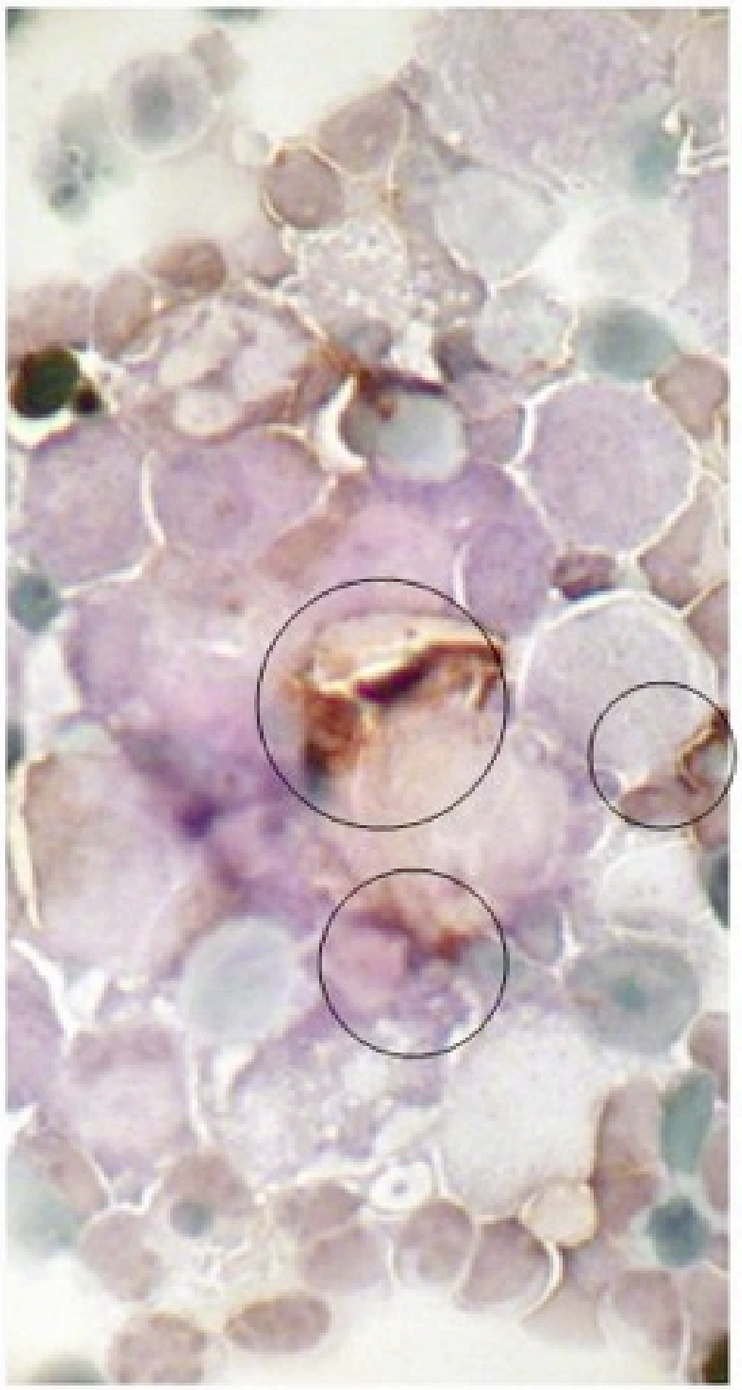


Control Culture



Bevacizu-mab



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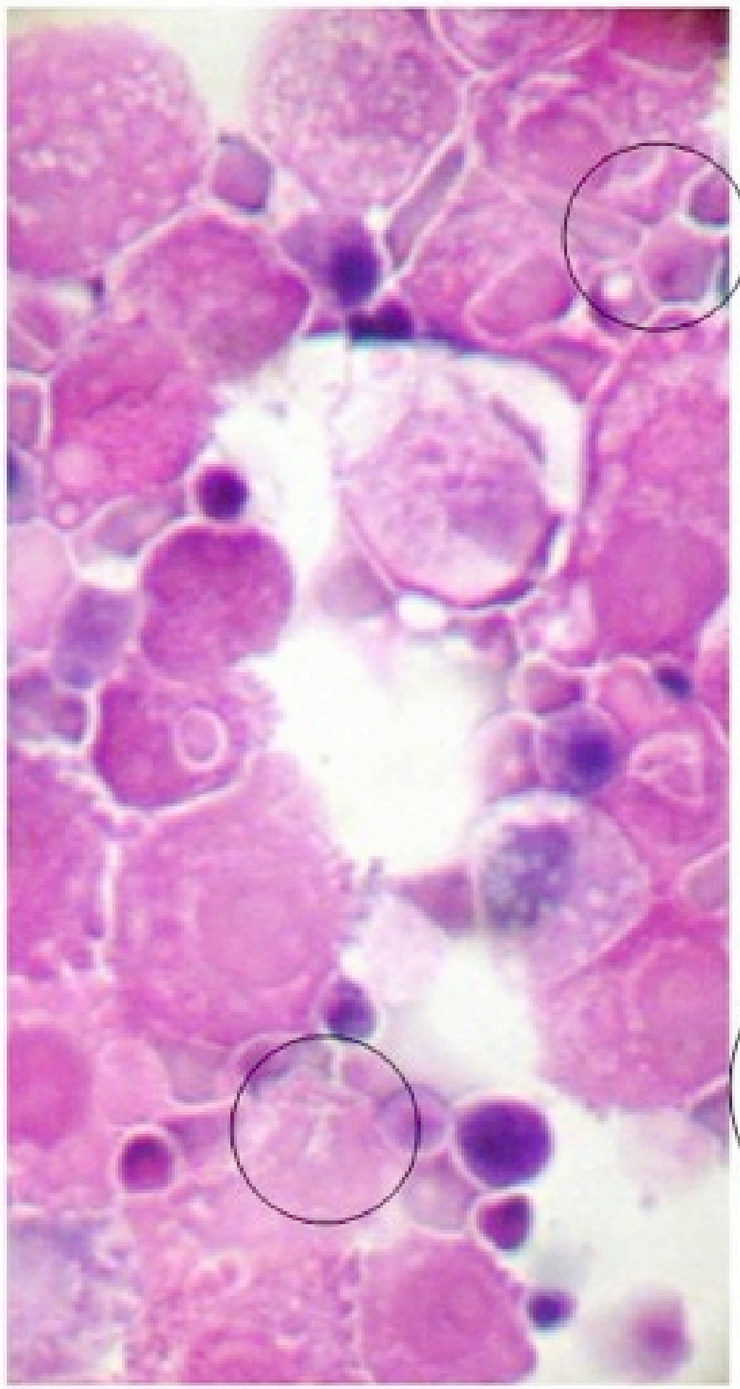

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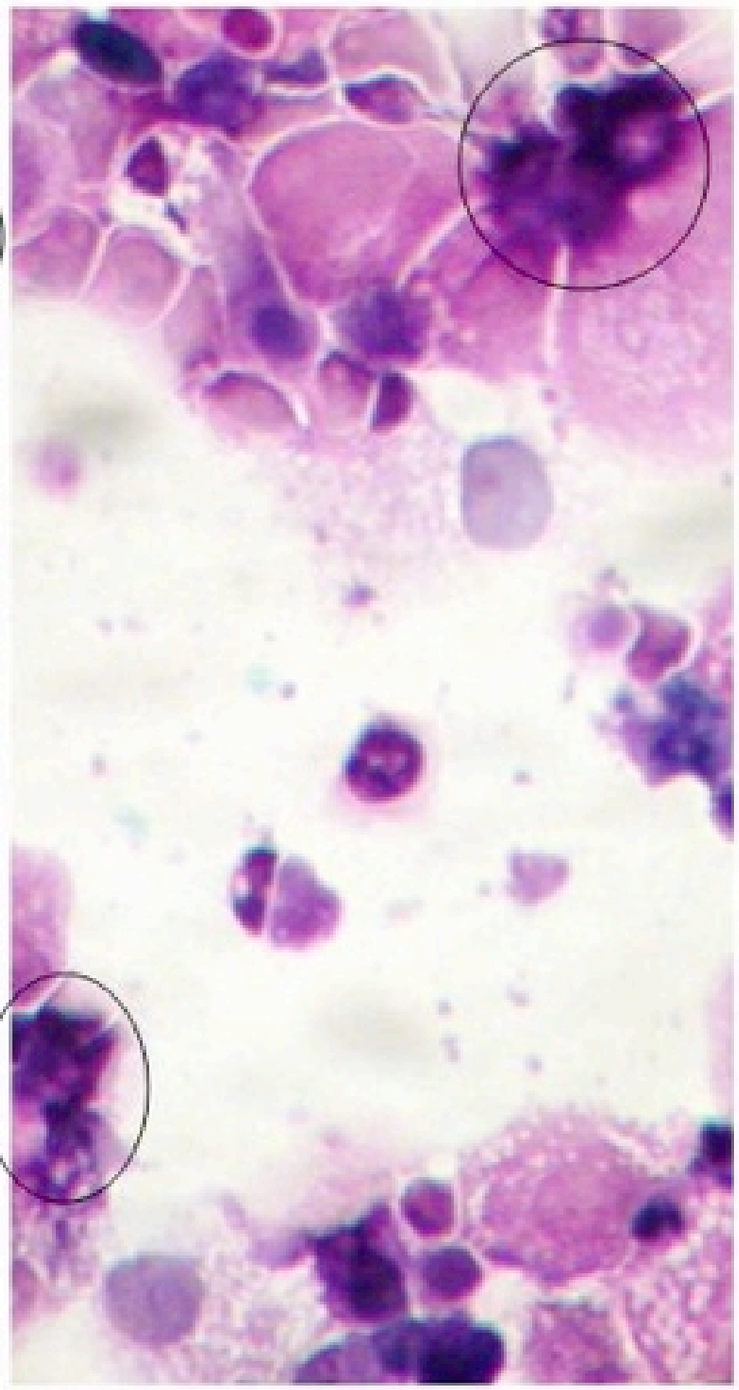
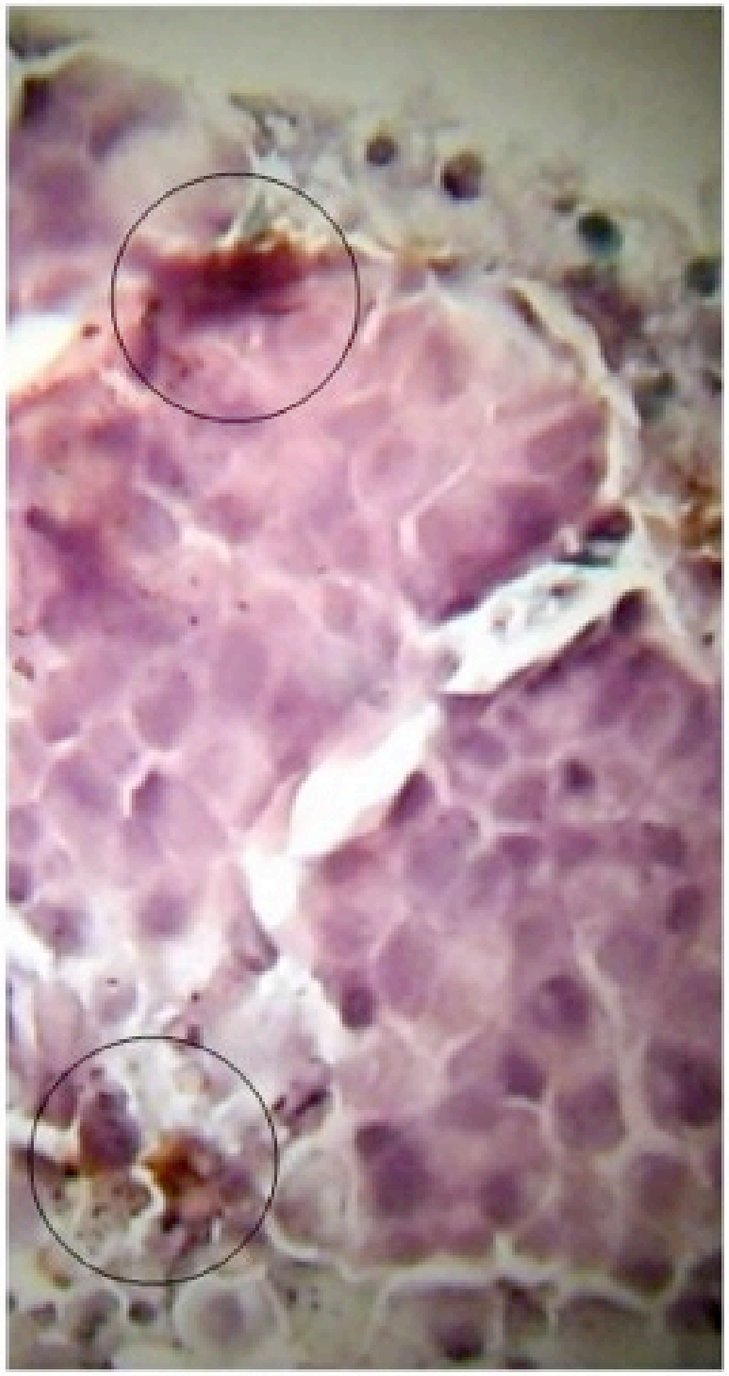


Bevacizu-mab

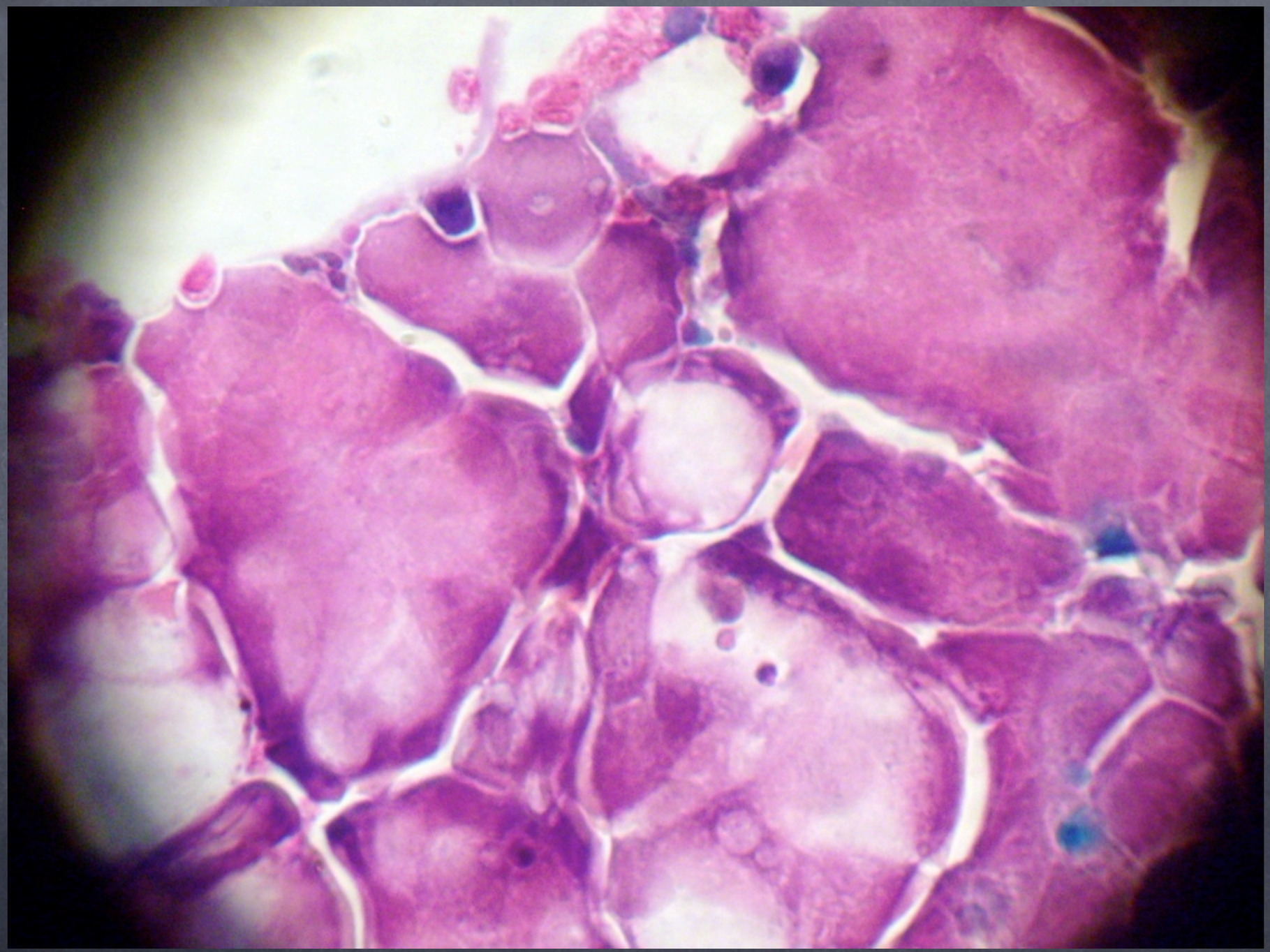


CD31

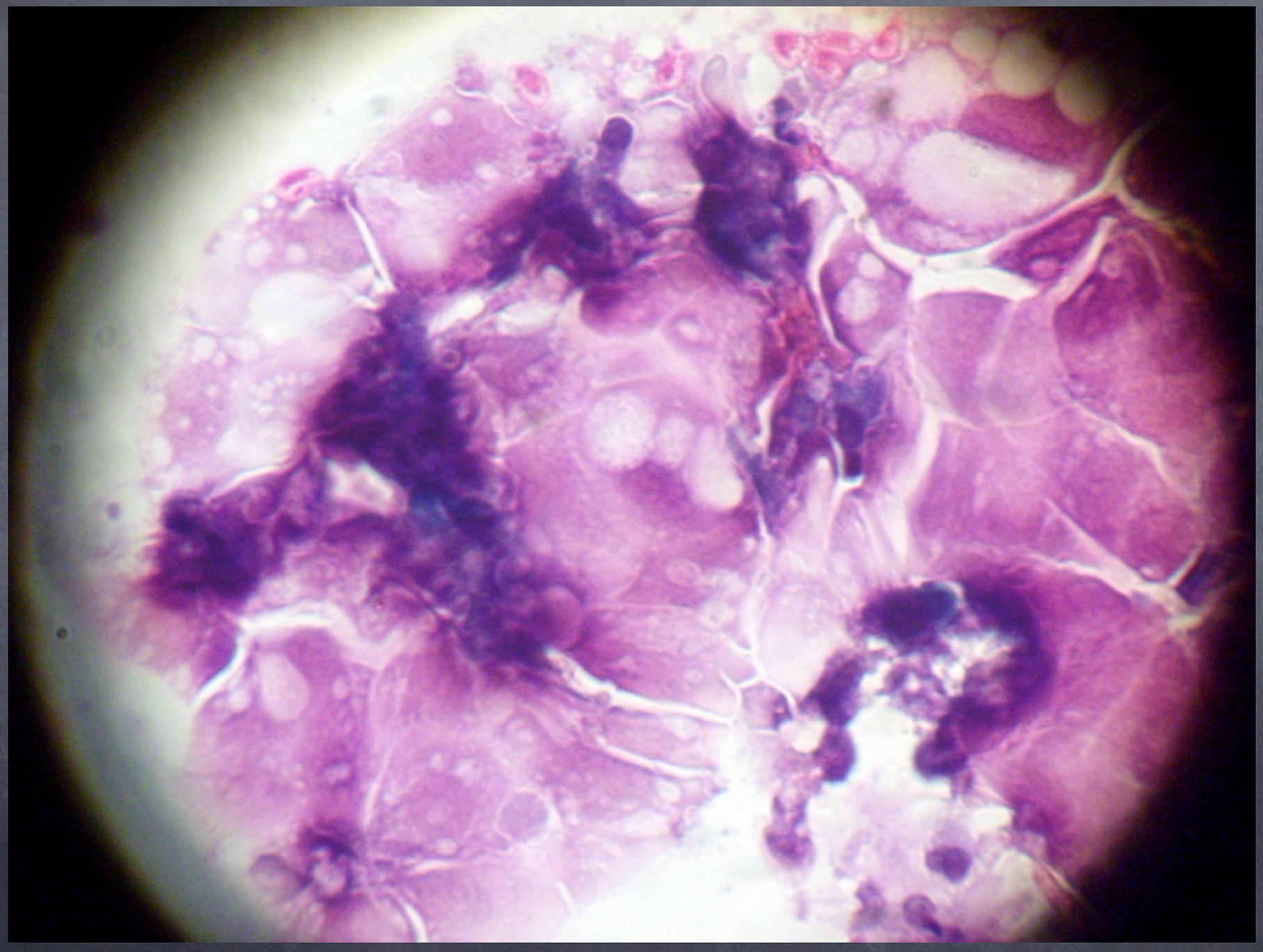
Control Culture

Bevacizu-mab

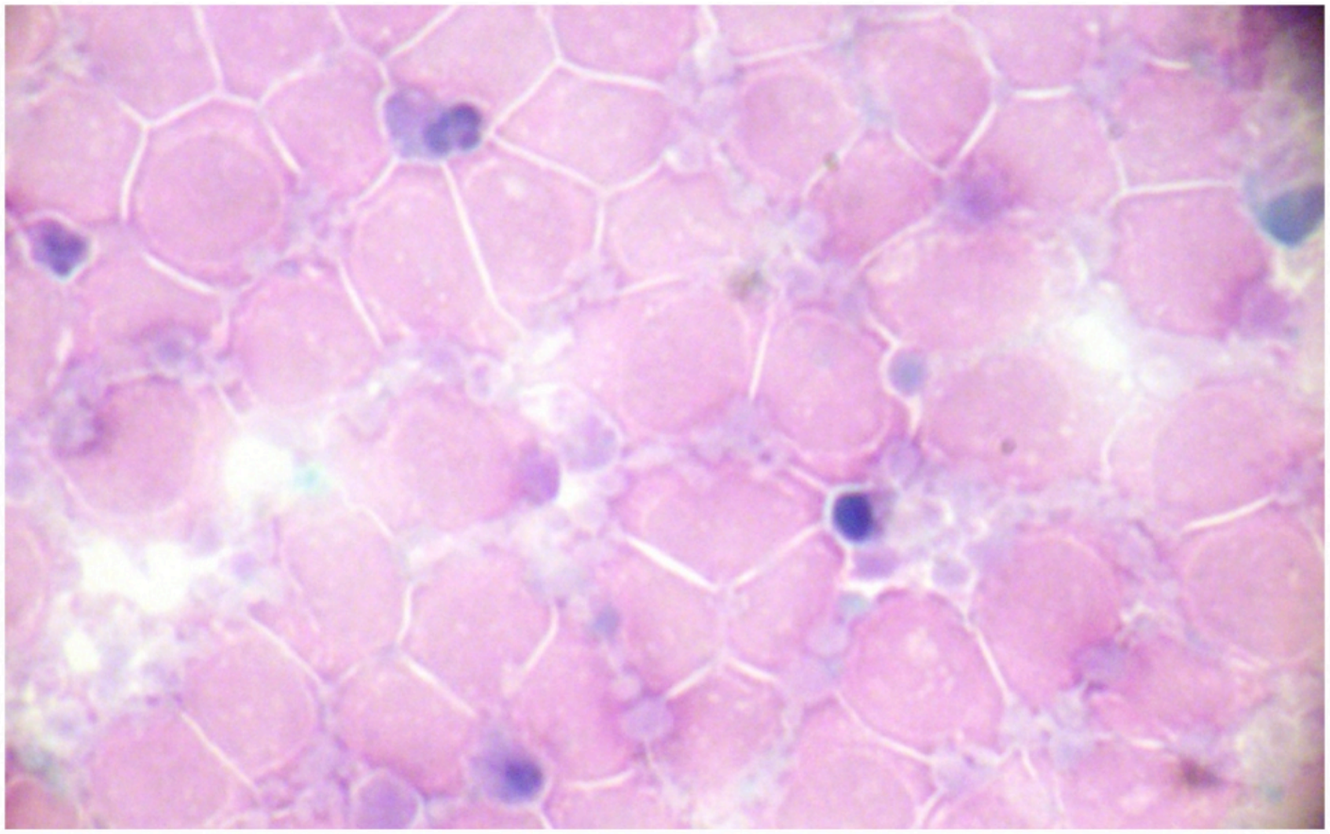
Ovarian Cancer, Control



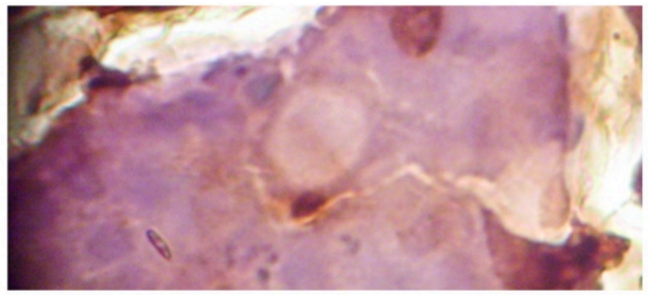
Ovarian Cancer, Erlotinib



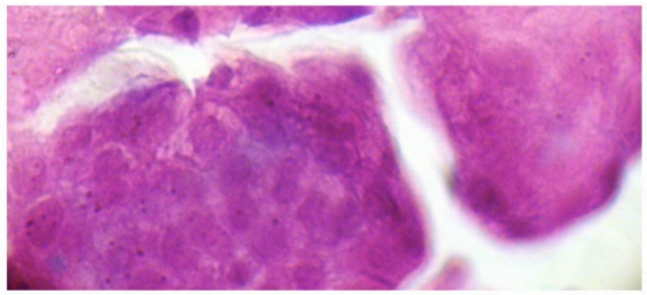
Bevacizumab, Merkel Tumor



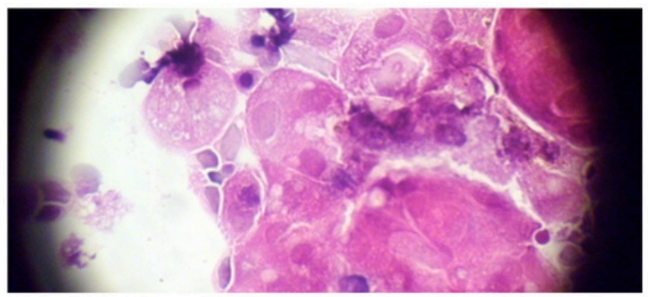
0 - 4+ Scoring System



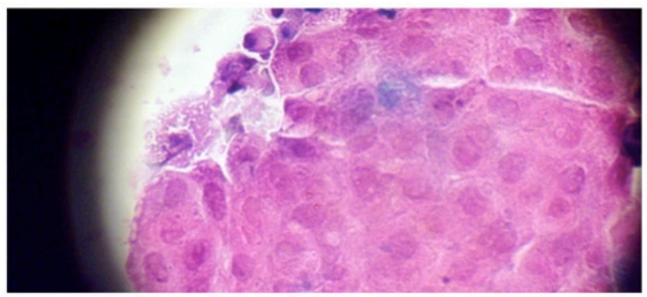
Mesothelioma CD31



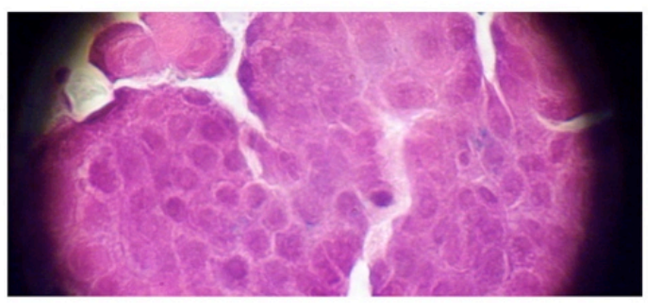
Control Culture 0+



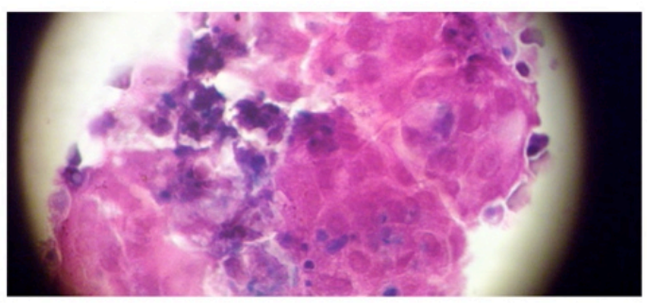
Sorafenib 3+



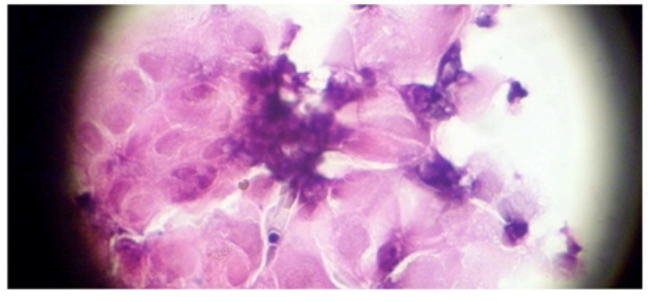
Sunitinib 1+



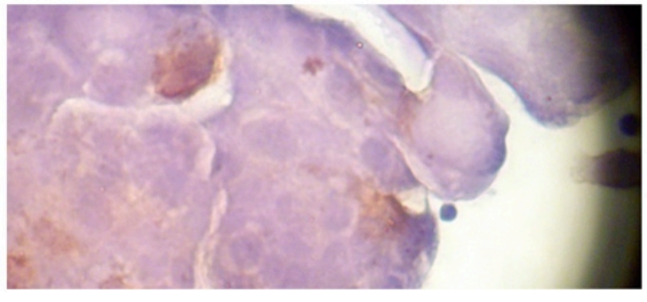
Gefitinib 1+



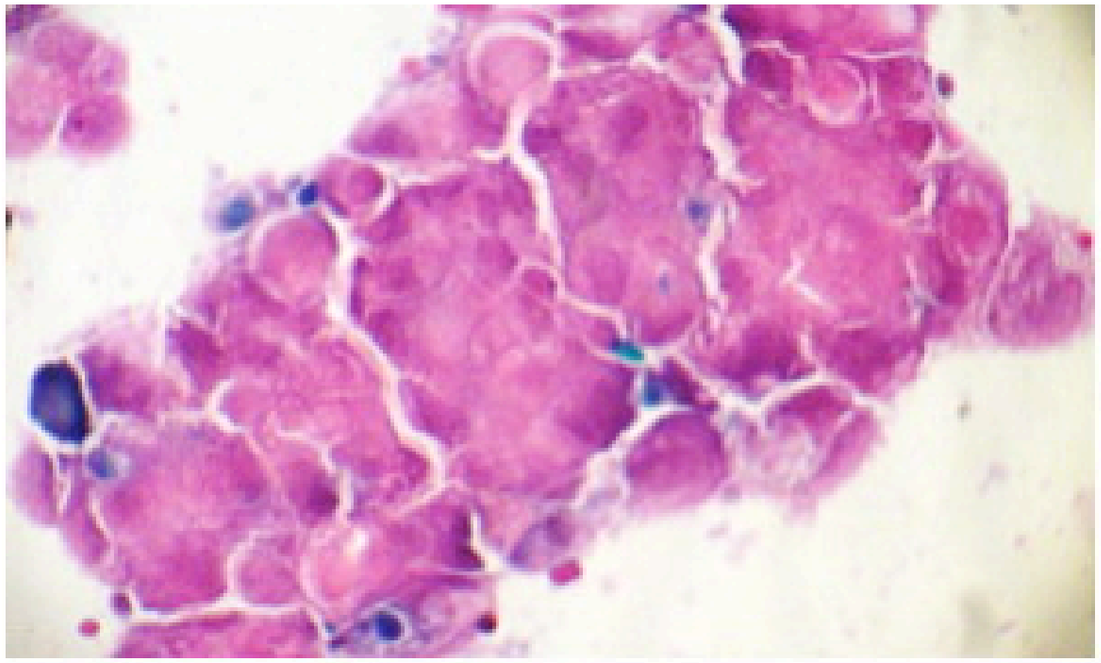
Erlotinib 4+



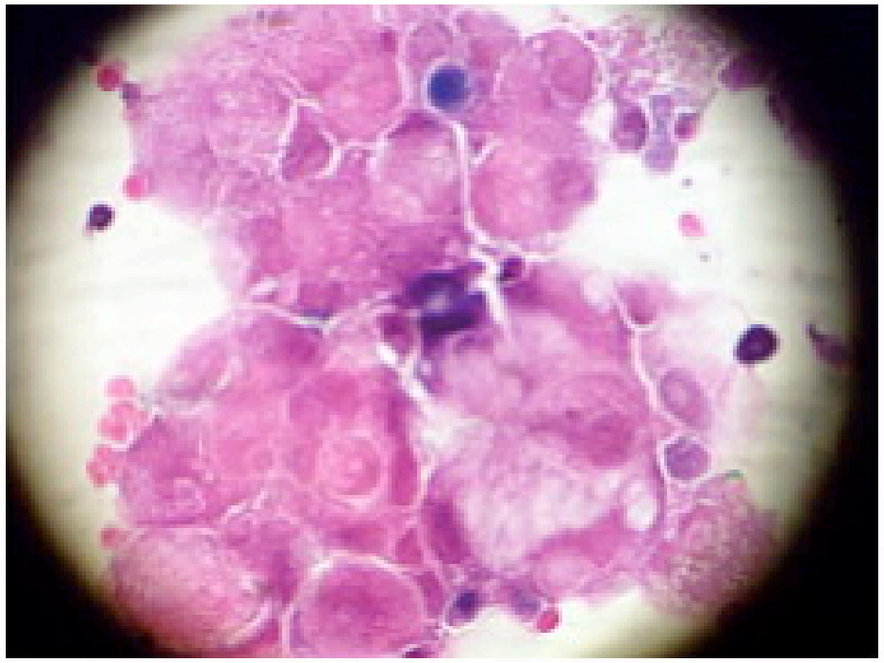
Bevacizumab 4+



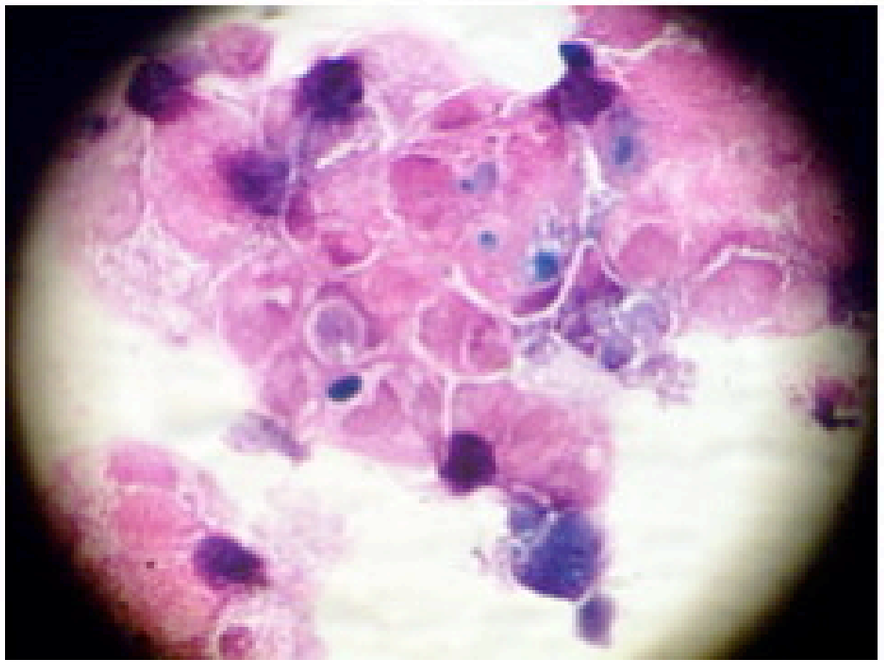
Mesothelioma CD31



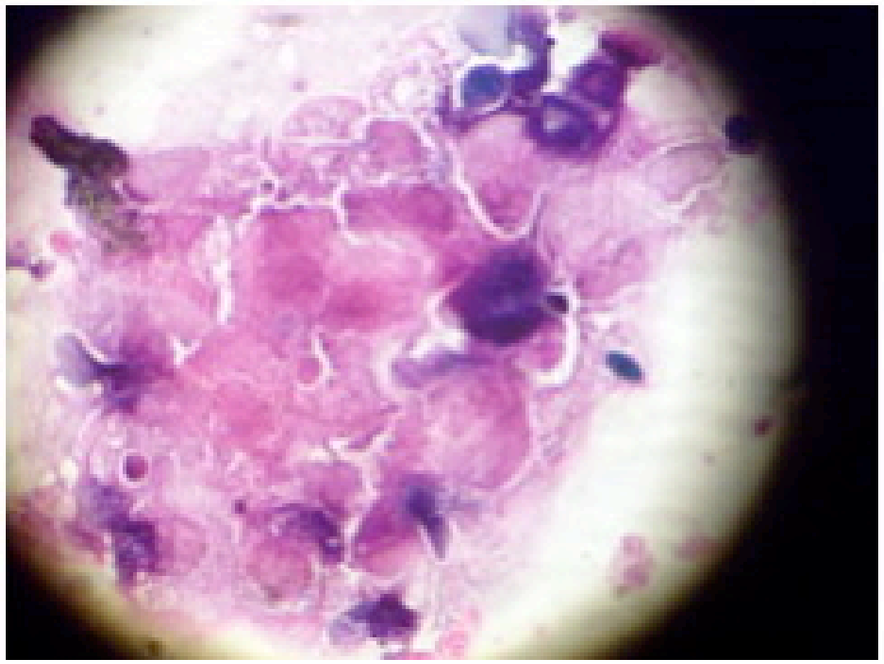
Control Culture



Erlotinib

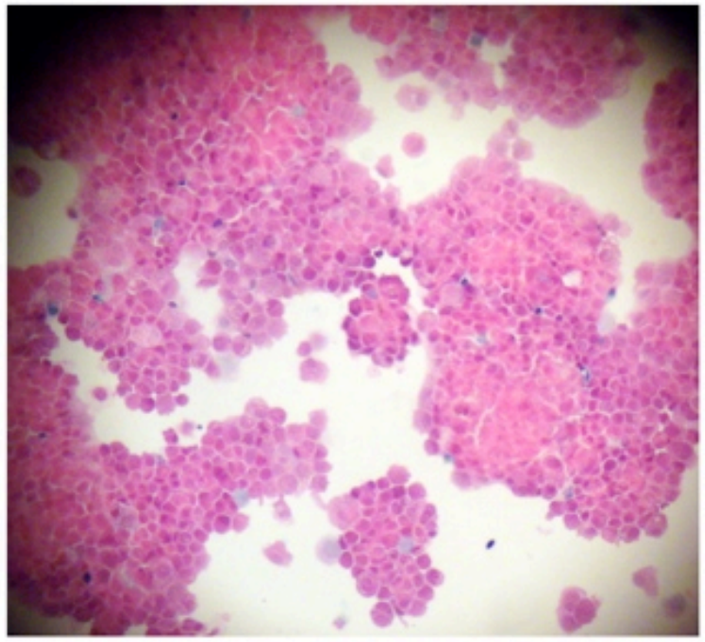


Bevacizumab

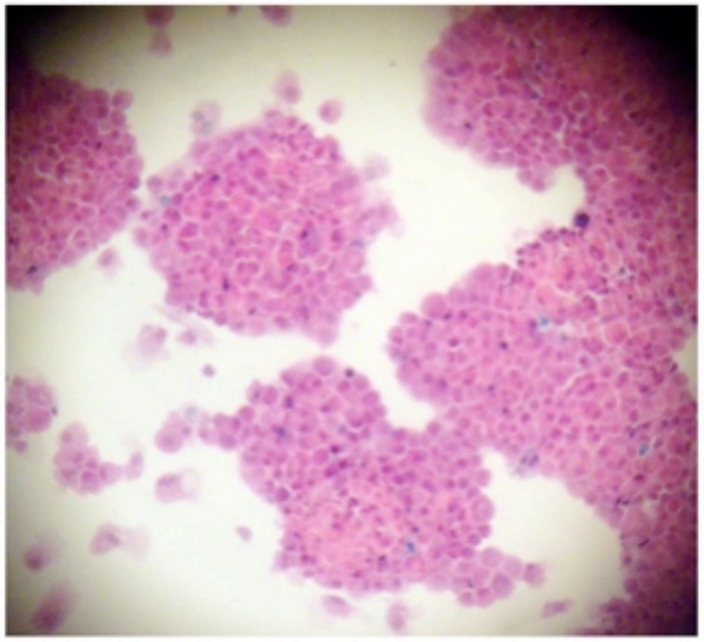


Bevacizumab + Erlotinib

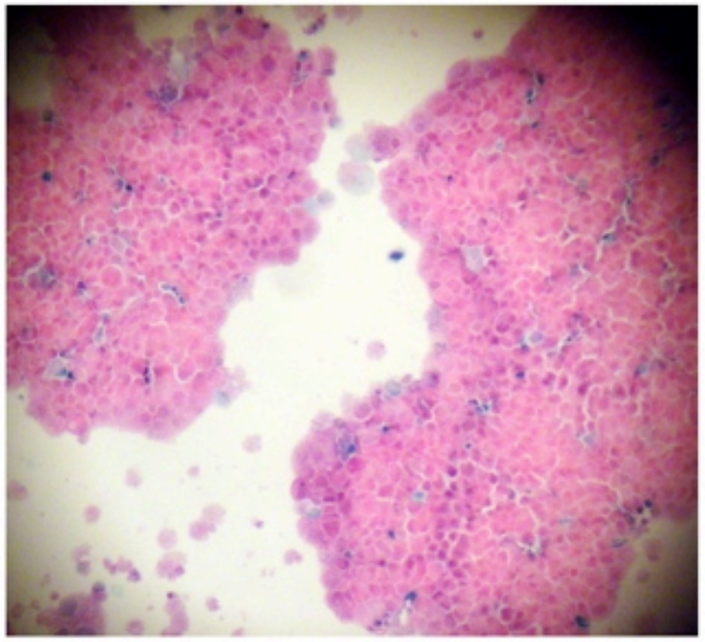
Anti-Microvascular Synergy



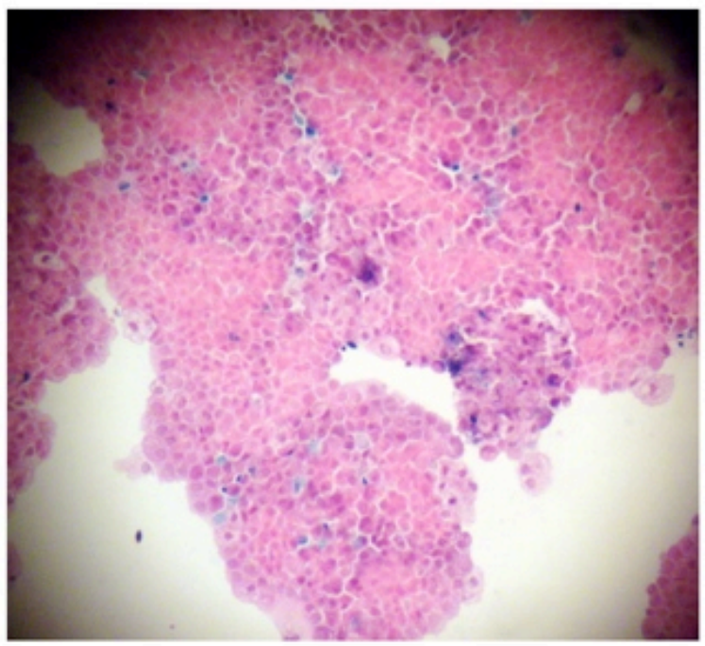
Control Culture



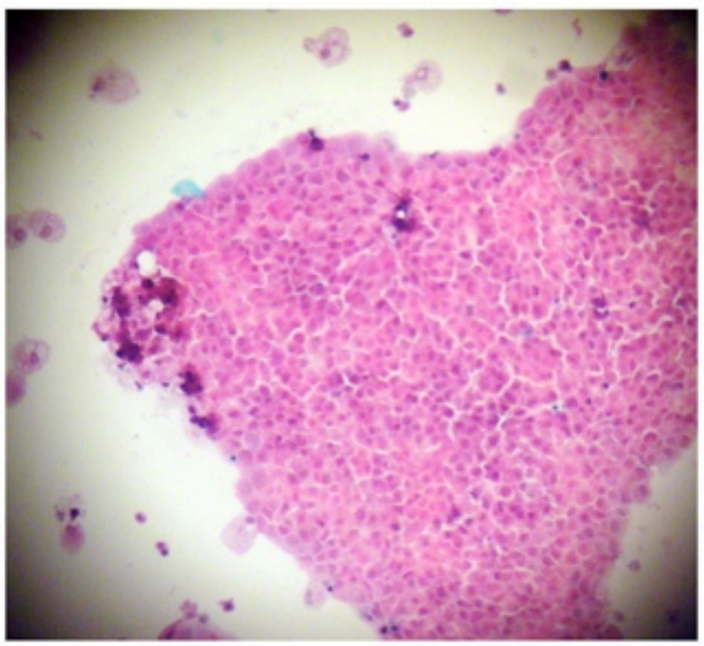
Erlotinib



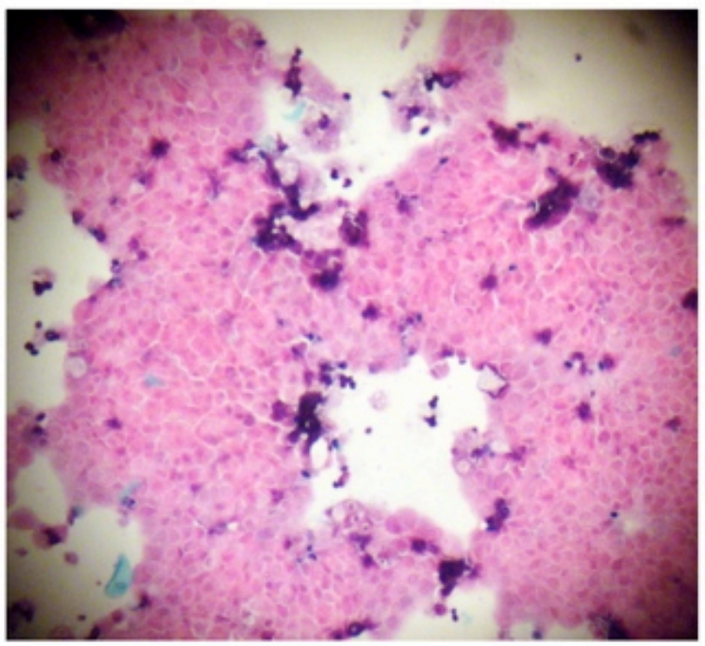
Imatinib



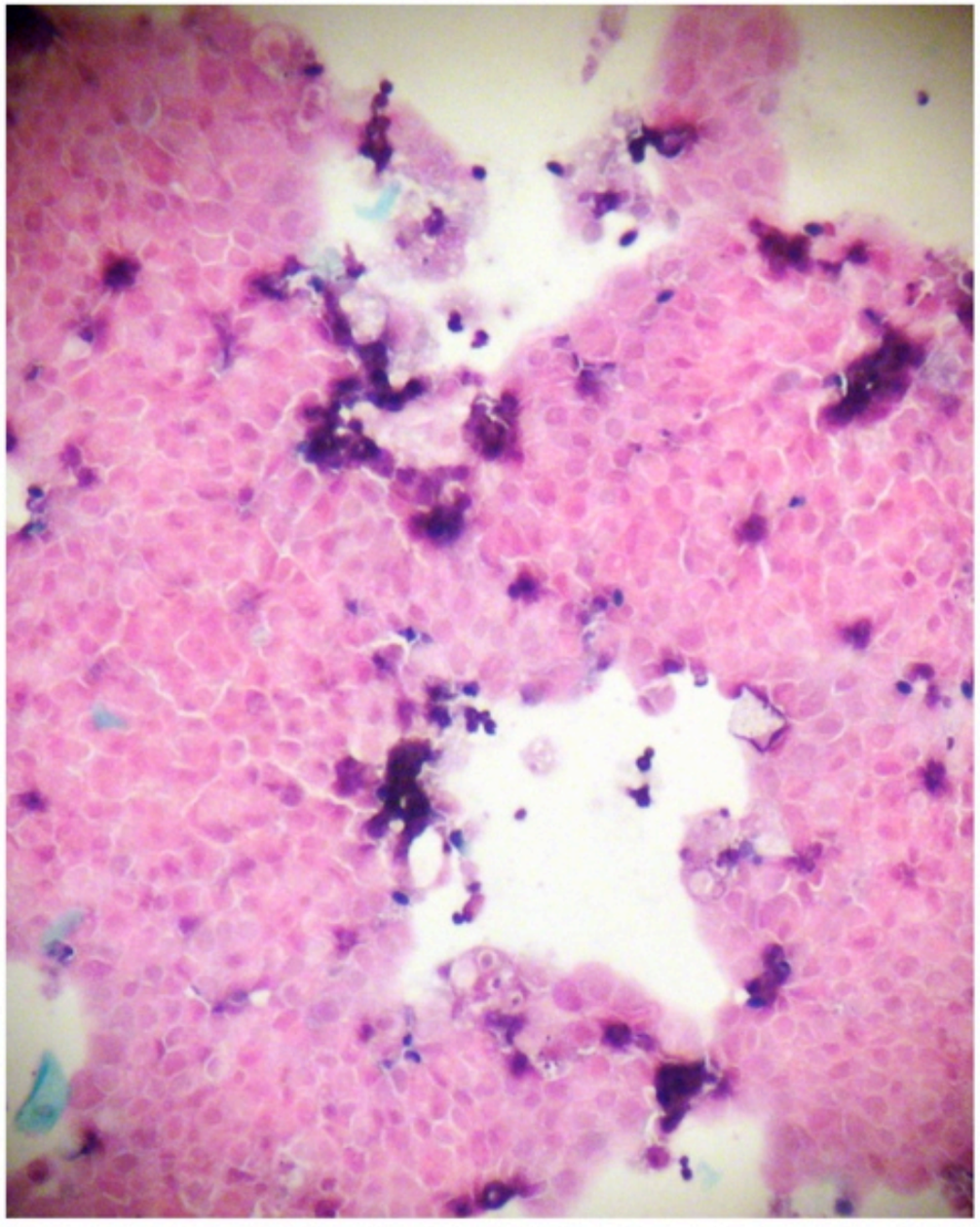
Bevacizumab



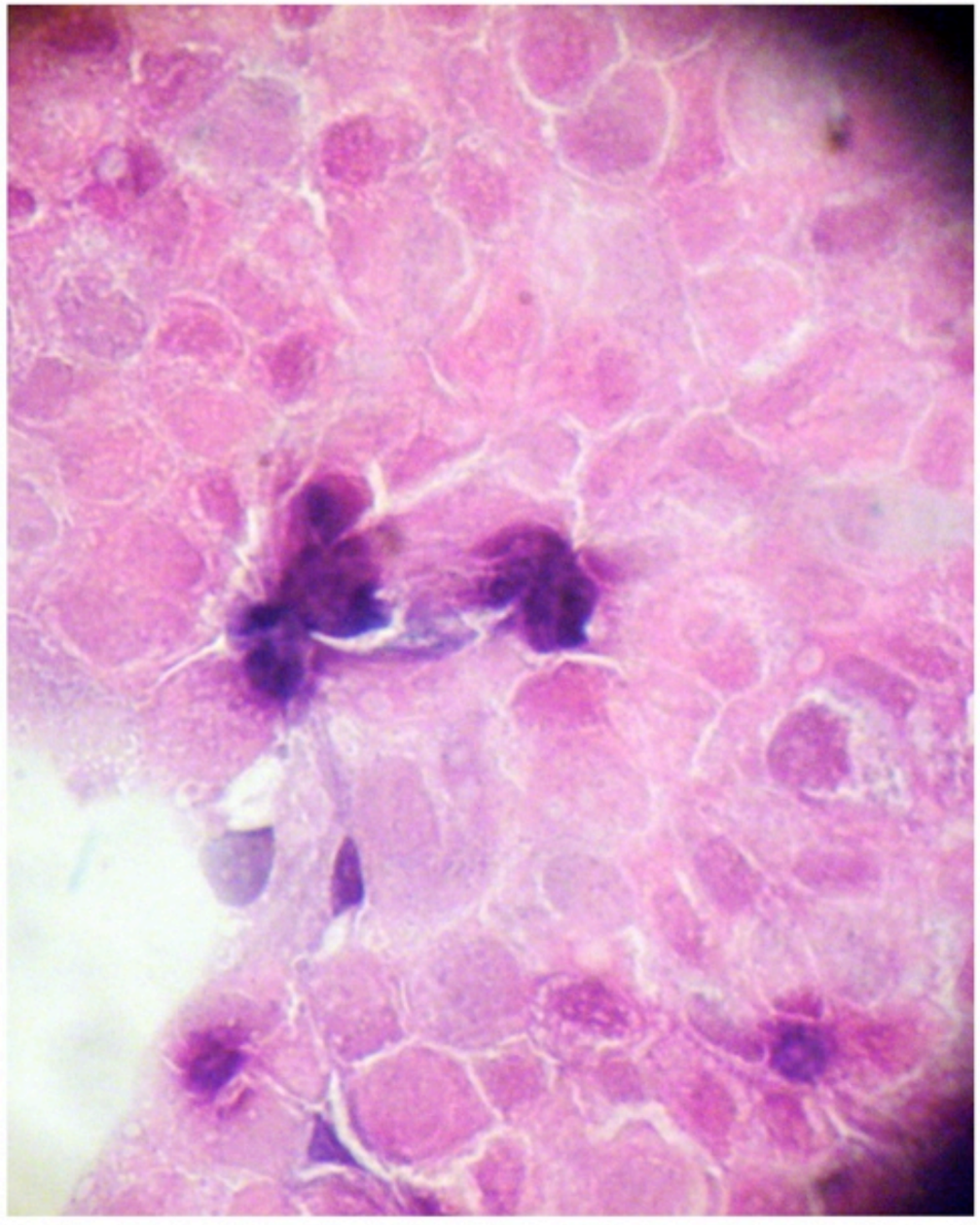
Erlotinib +
Bevacizumab



Imatinib +
Bevacizumab

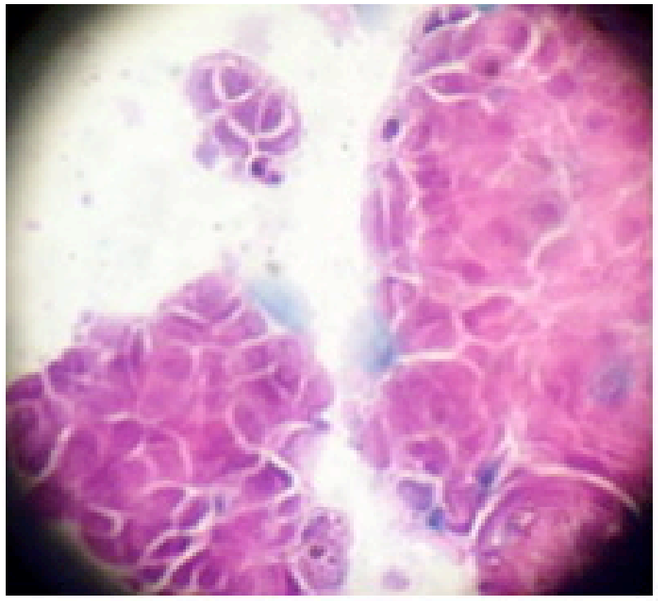


Imatinib + Bevacizumab
100X

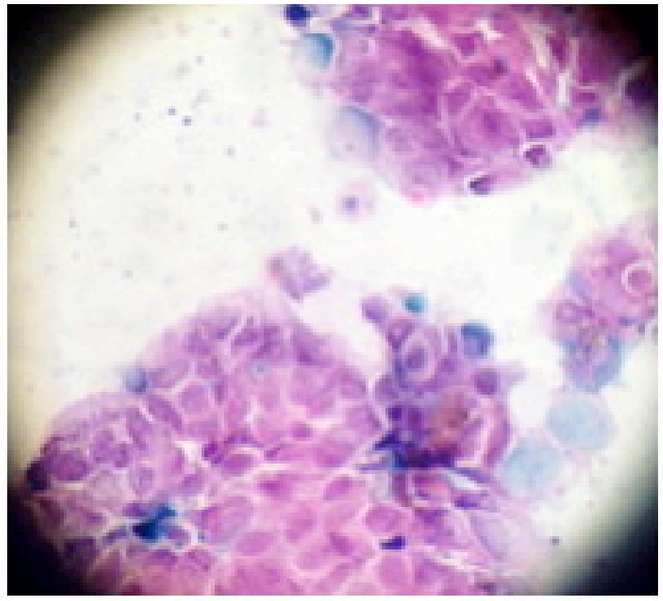


Imatinib + Bevacizumab
400X

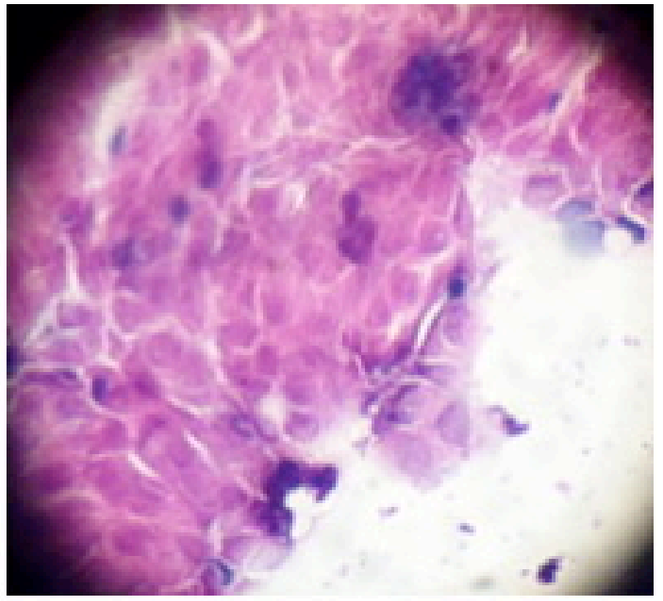
True "Functional Profiling"



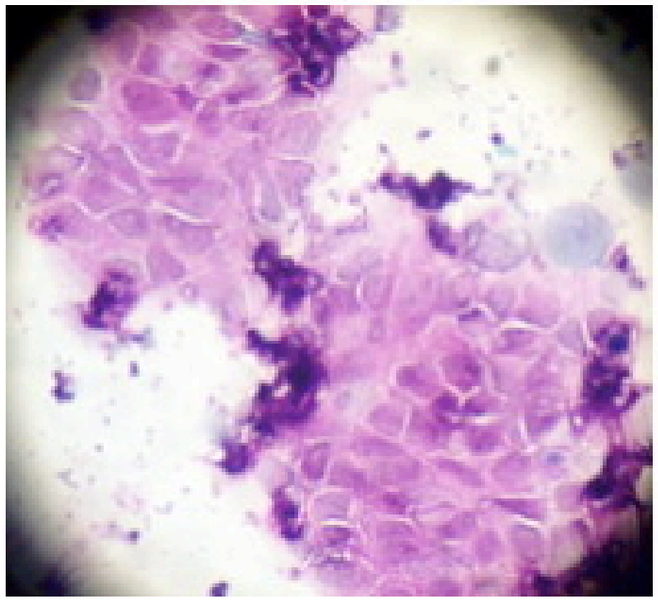
Control Culture
(Carcinoid)



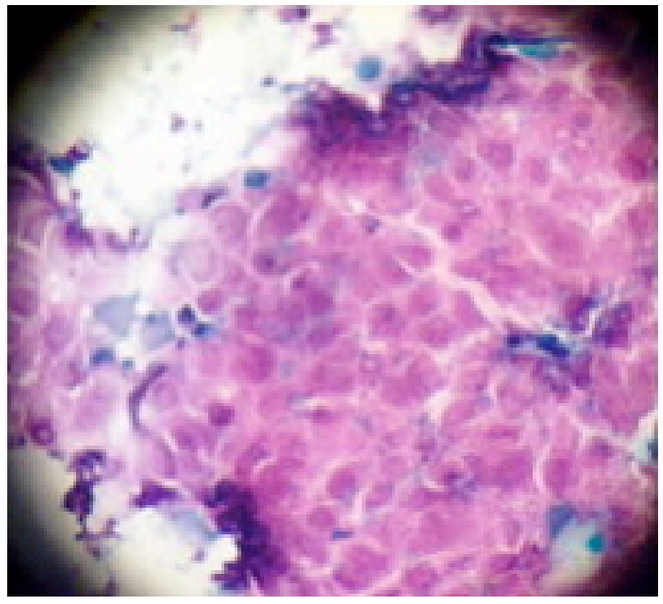
Sorafenib



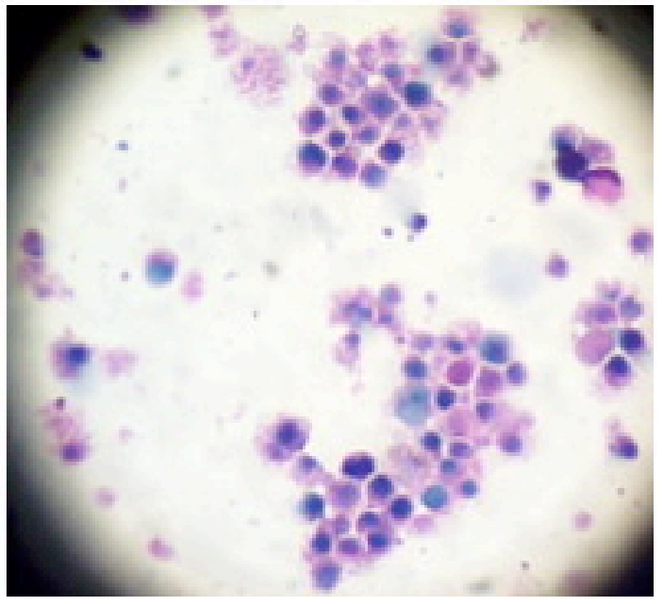
Erlotinib



Bevacizumab

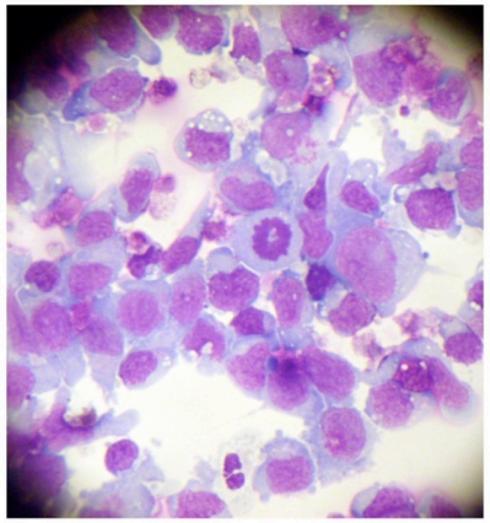


Erlotinib +
Bevacizumab

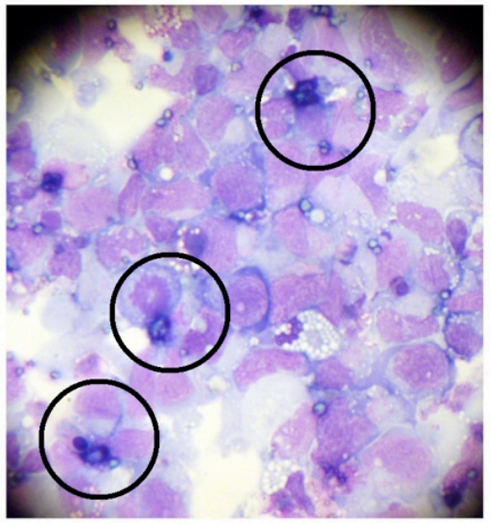


Sunitinib

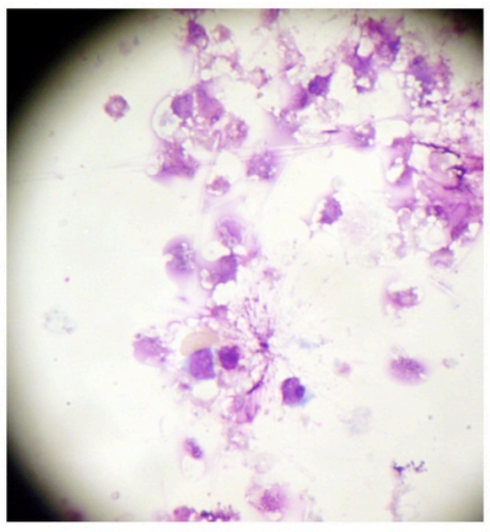
7 Day Control Duplicate Culture



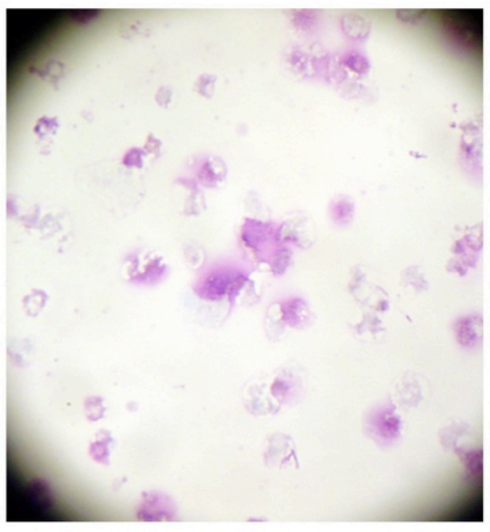
Bevacizumab



Gefitinib



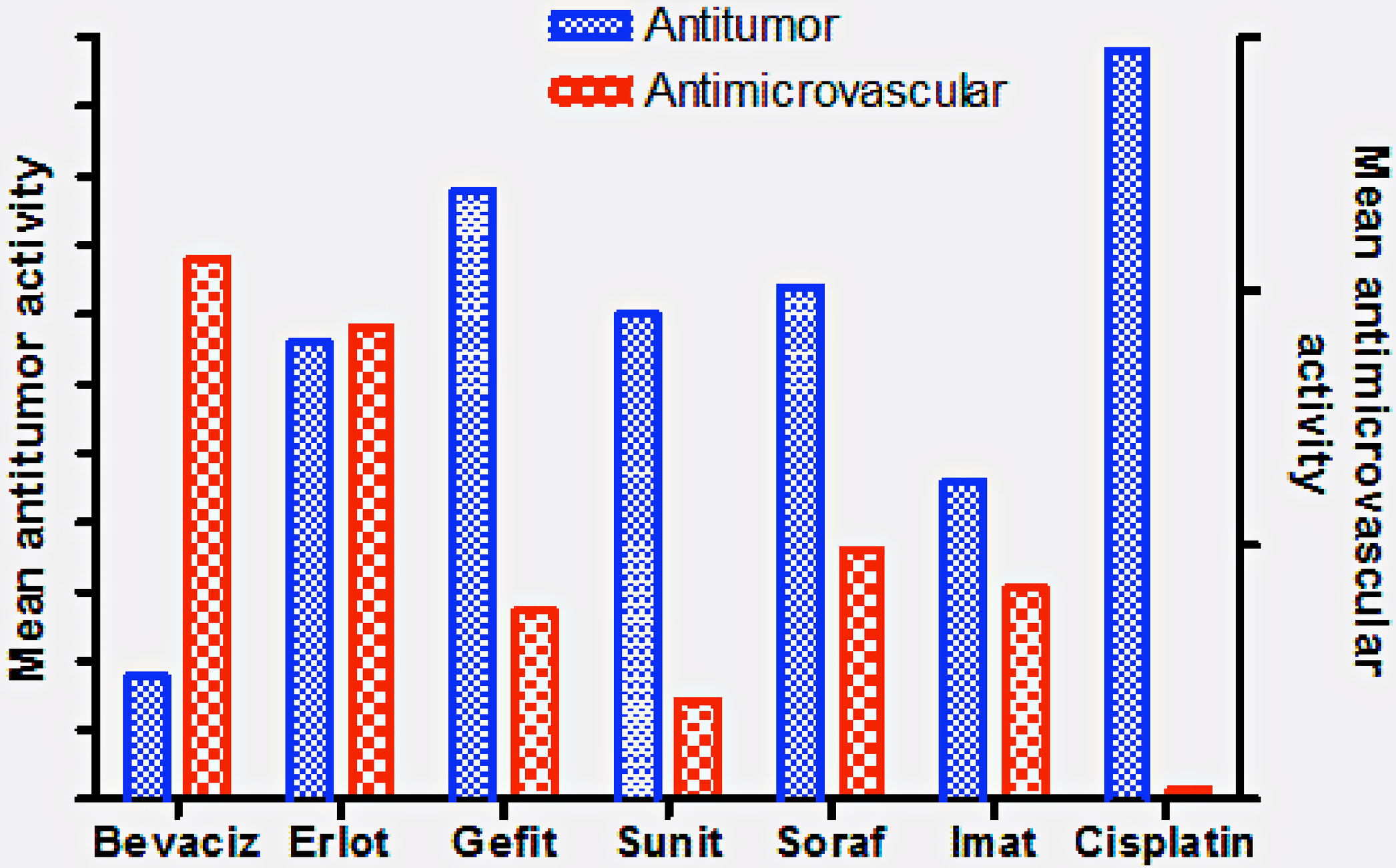
Erlotinib



ANLL Endothelial Cells

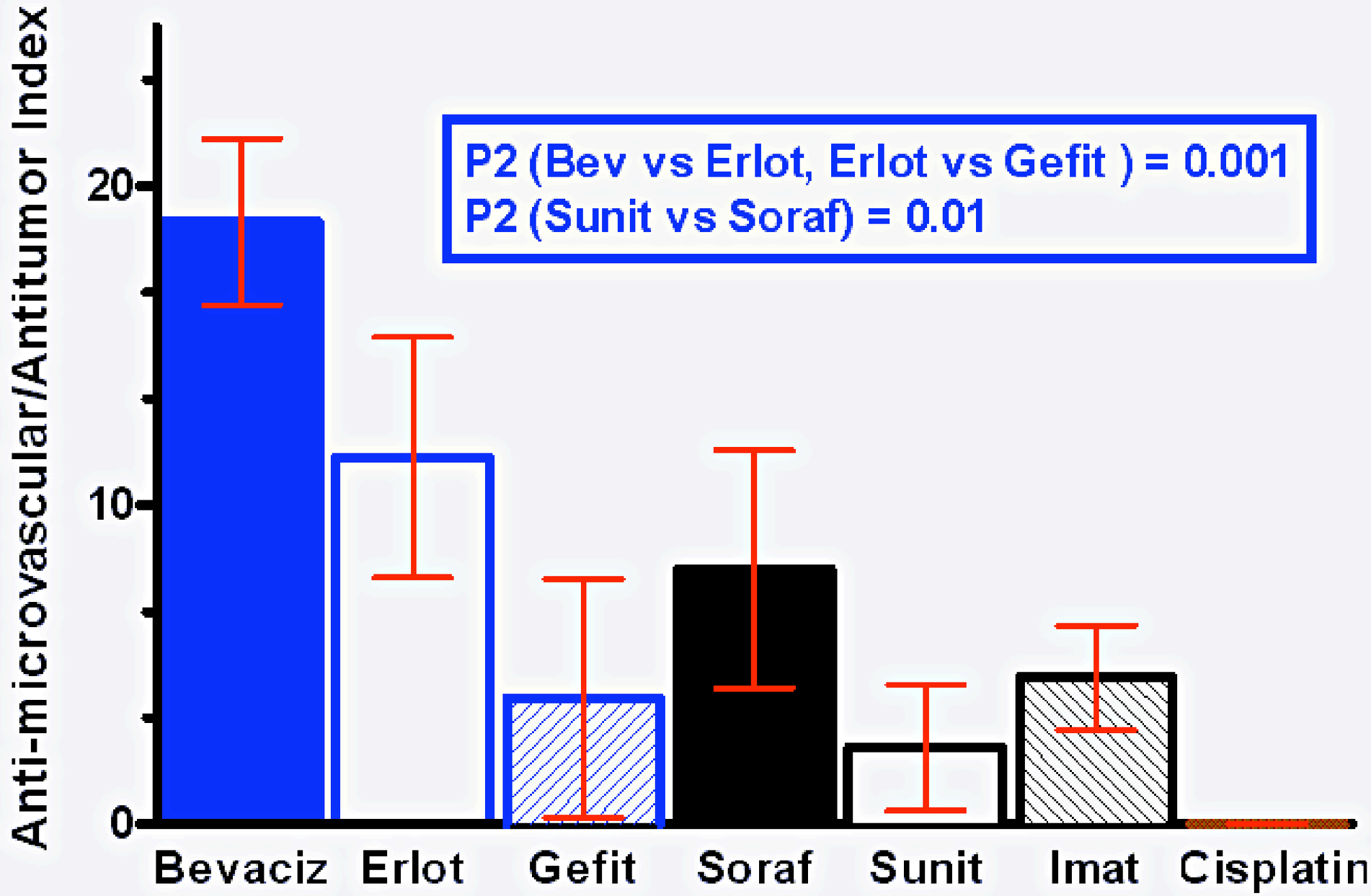
Antitumor versus antimicrovascular activity

n = 50 to 100 paired comparisons between antimicrovascular and antitumor activity



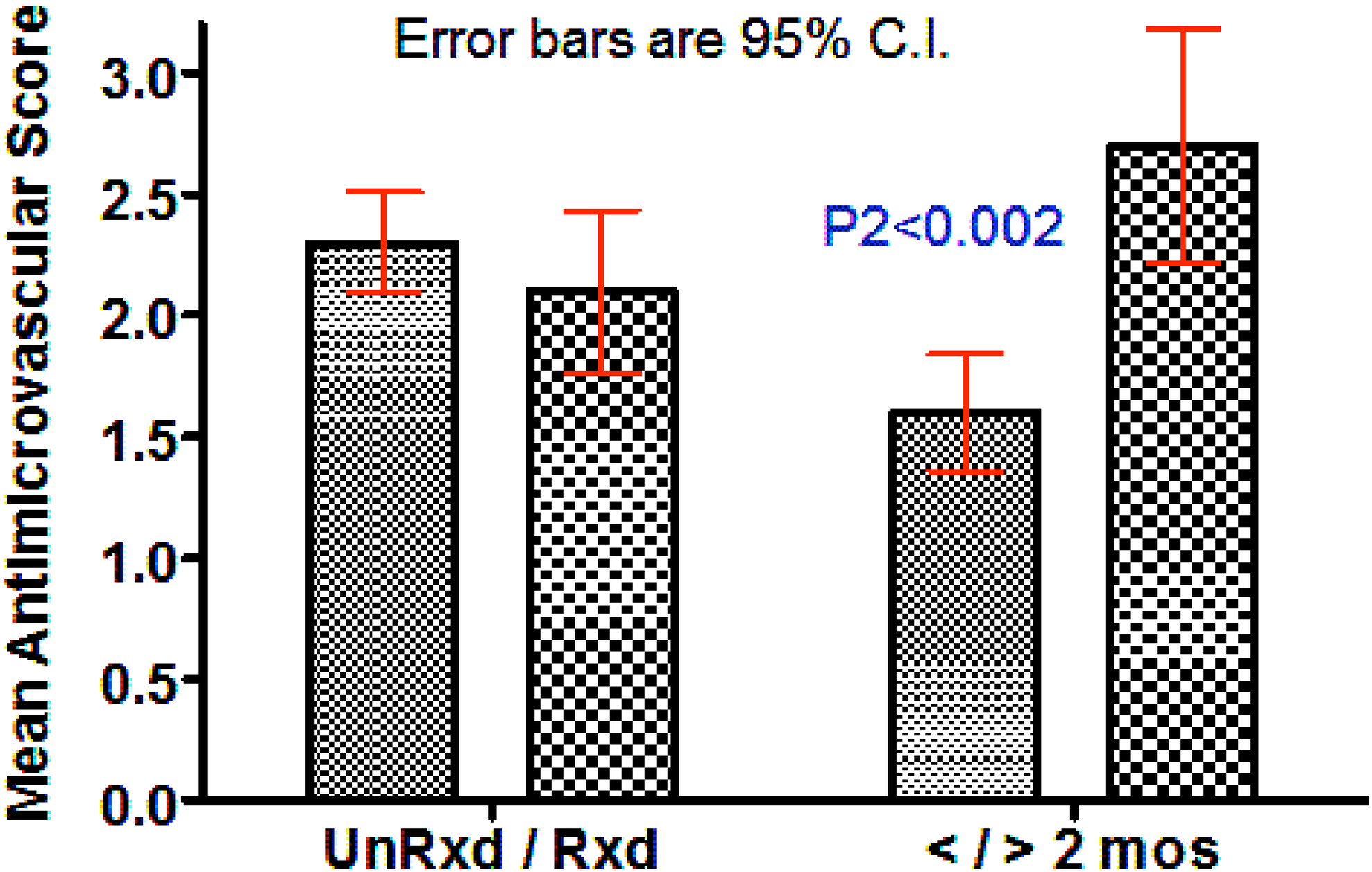
Anti-microvascular activity relative to anti-tumor activity

Error bars 95% C.I.



P2 (Bev vs Erlot, Erlot vs Gefit) = 0.001
P2 (Sunit vs Soraf) = 0.01

Bevacizumab Activity as a function of treatment status



Antimicrovascular effect of bevacizumab in previously treated tumors as a function of time since most recent chemotherapy

Last Chemo Avastin Effect	< 2 months Low	< 2 months High	> 2 months Low	> 2 months High	P2 =
All Treated	19	16	9	27	0.016
All AdenoCa	16	10	6	20	0.011
Breast,Colon, NSCLC,Ovary	14	7	5	19	0.003
Breast	4	3	2	5	0.59
Colon	2	1	1	3	0.49
NSCLC	5	0	1	6	0.015
Ovary	5	1	1	5	0.080

What is the *best* endpoint?

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

Clinical Relevance

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.