NEW THERAPEUTIC DEVELOPMENT APPROACHES TO HUMAN CANCERS:

Target ID - Target Validation in Pathogenesis - Evaluation of Therapeutic Approaches and Combinations - Clinical Application

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THE PAST

The “One-Size-Fits-All” Approach to Cancer

Traditional Clinical Approaches to Initial Malignancy

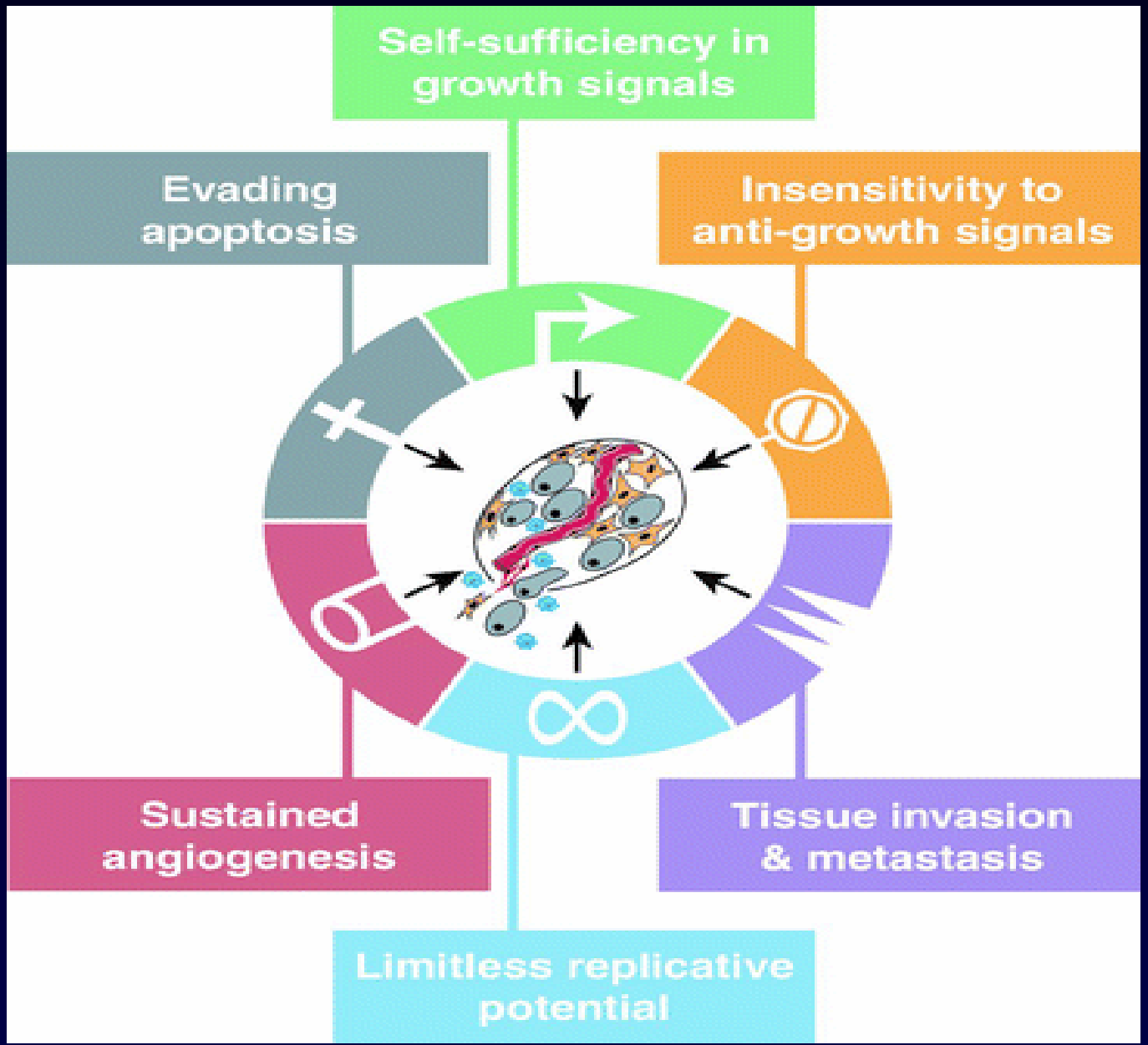
- SURGERY - Traditional excisional approaches with clean margins i.e. “we got it all”. Newer approaches include cryosurgery, hyperthermic surgery, radiofrequency ablative surgery, etc.

- RADIATION THERAPY - Traditional external beam, IMRT, brachytherapy (implants)

- SYSTEMIC THERAPY - Cytotoxics (chemotherapy), hormonal therapy, biologic therapy

We Need a Paradigm Shift - A New Approach Based on the Biology of the Disease

- Premise #1 - Cancer is not a single disease.
- Premise #2 - Cancer is not a single disease even within a given histology. The only thing ALL breast cancers share in common is that they arise in the organ that defines us as a species - the breast.
- Premise #3 - A need to develop new therapeutic approaches that take into account #1 and #2



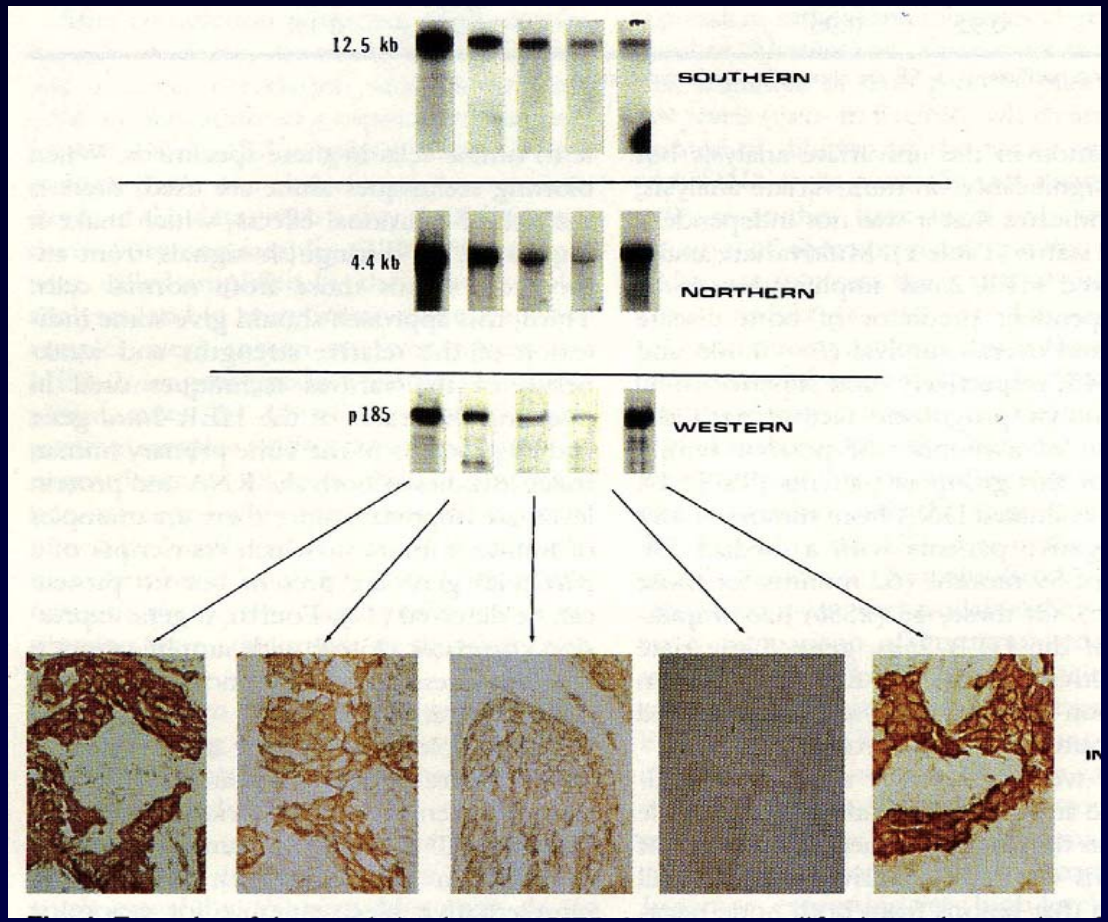
Hanahan and Weinberg Cell 2000

Lessons from the HER2 Story

- 1.) Target Identification
- 2.) Target Validation
- 3.) Preclinical Confirmation
- 4.) Determintion of Potential Usage Preclinically
- 5.) Clinical Translation - Proof of Concept
- 6.) Clinical Optimization

Target Identification

The HER2 Alteration



Southern

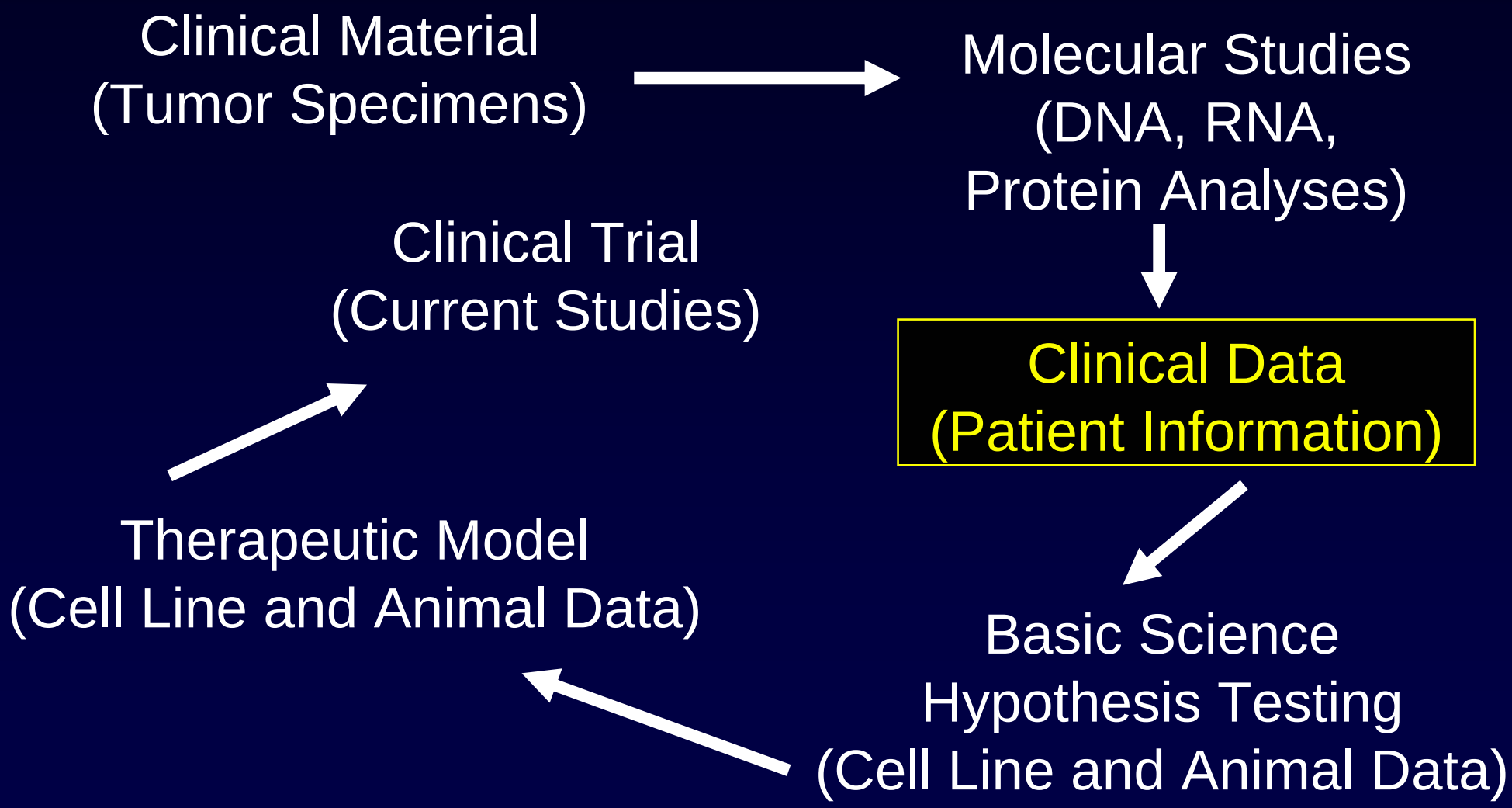
Northern

Western

IHC

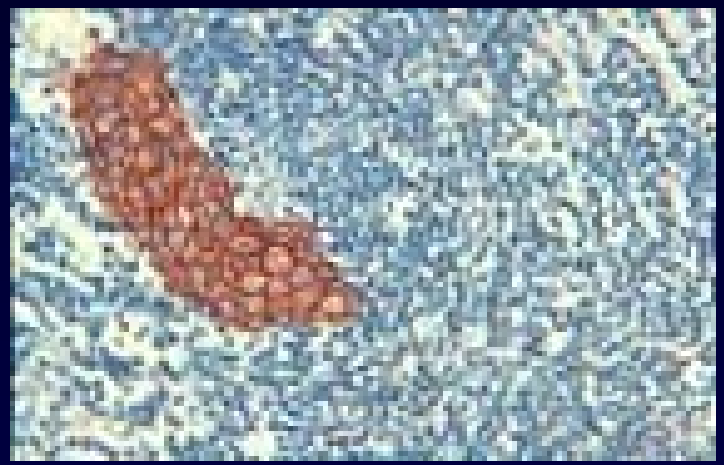
Slamon et al. *Science* 1989

HER-2/neu Program at UCLA



HER-2 Oncogene
Amplification

Breast Cancer



HER-2 Oncoprotein
Overexpression

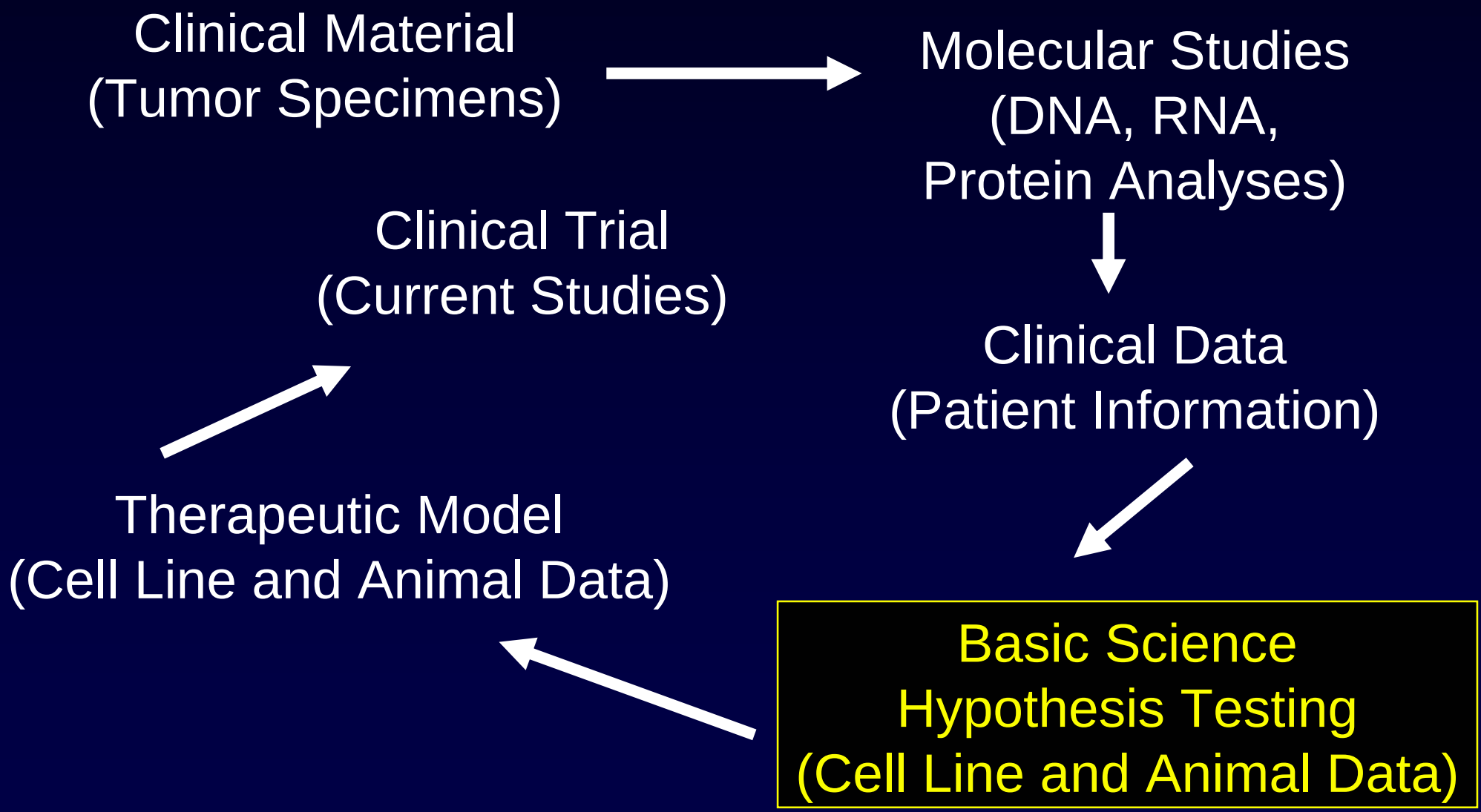
Shortened Survival

Median Survival from First Diagnosis

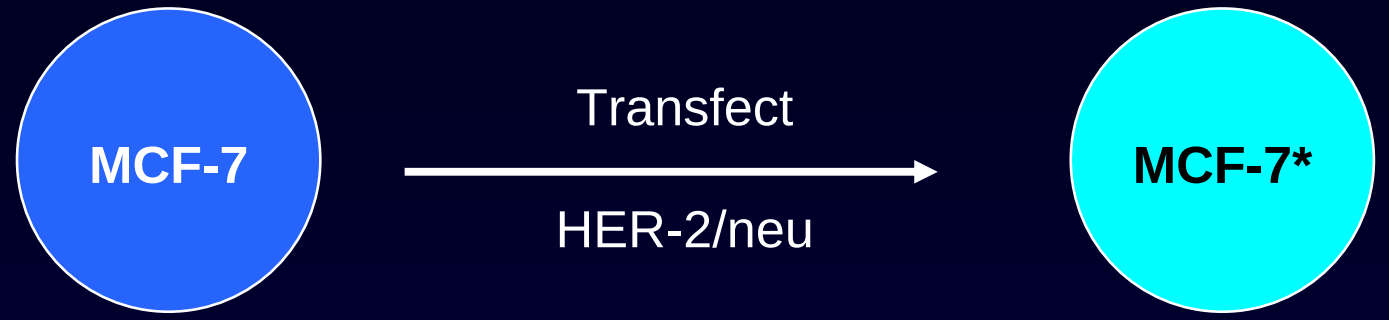
HER-2 overexpressing	3 yrs
HER-2 normal	6 - 7 yrs

Slamon et al, 1987

HER-2/neu Program at UCLA



Human Breast Cancer Cells



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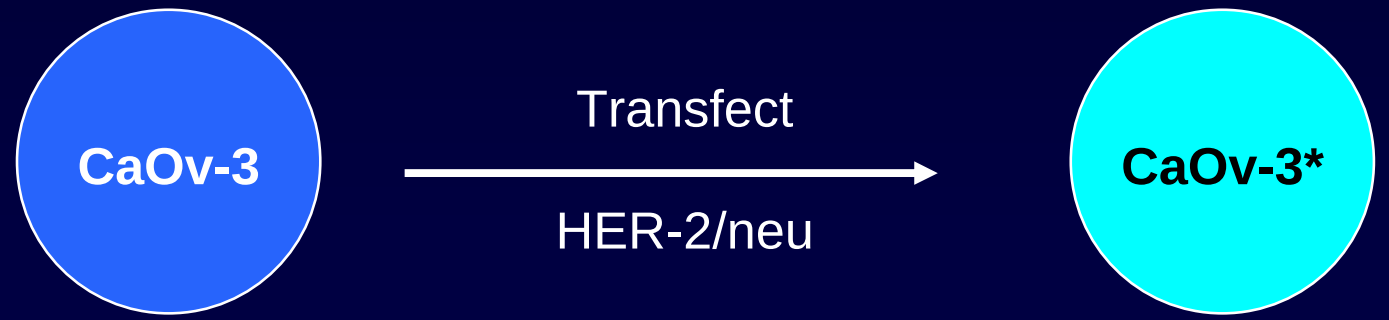
Human Ovarian Cancer Cells



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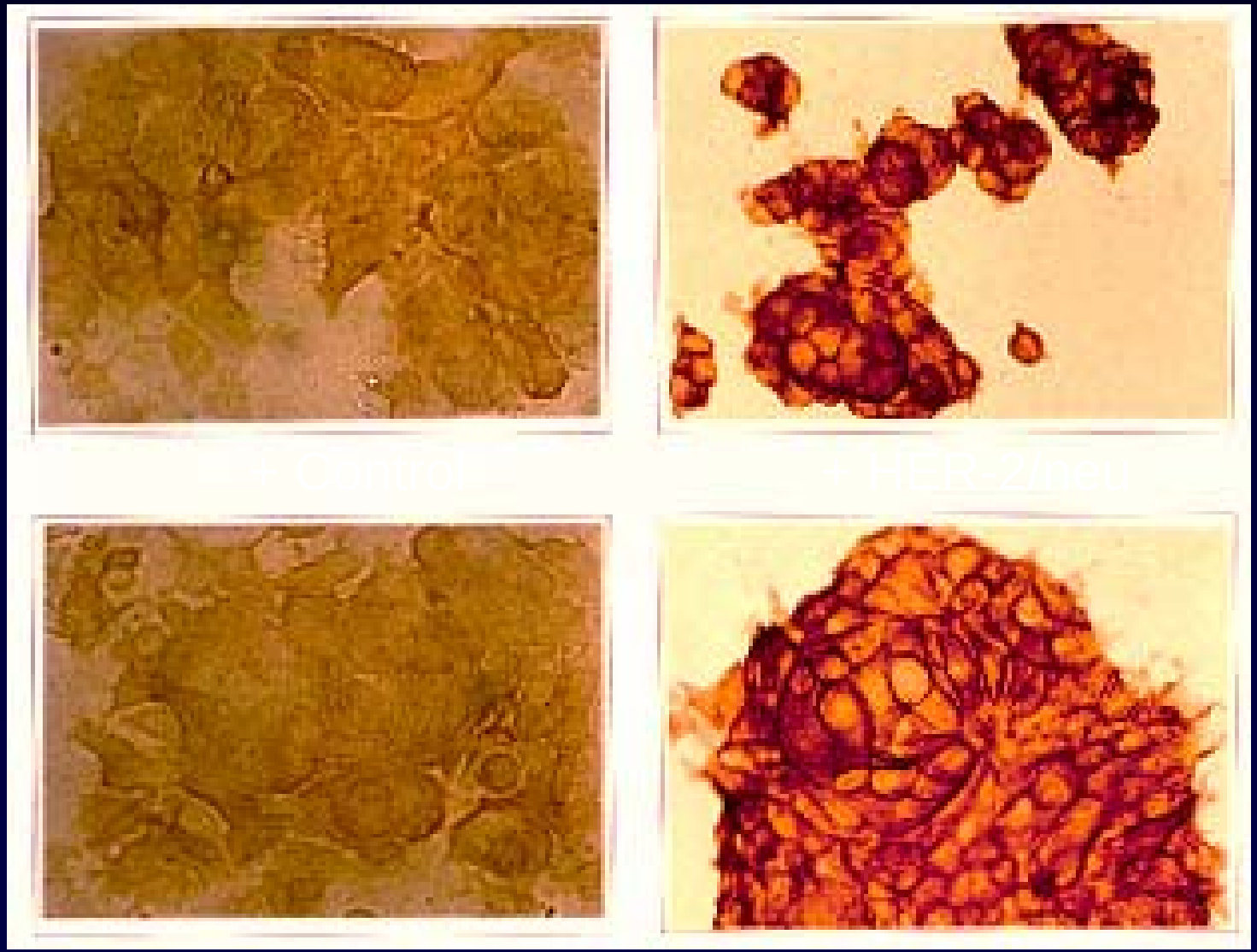
High Expressor

***Consistent results in 9 additional Breast & Ovarian Cancer Cell Lines**

Immunohistochemistry

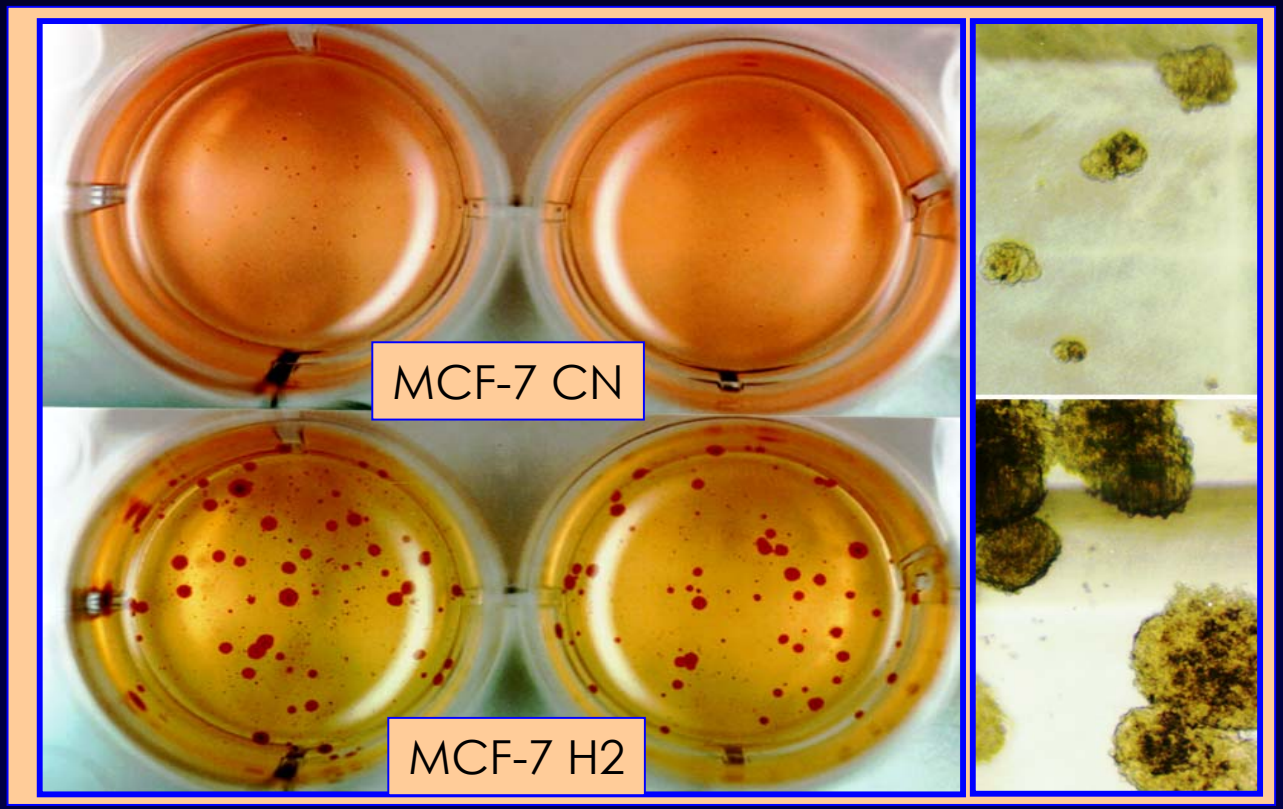
MCF 7

CaOV 3

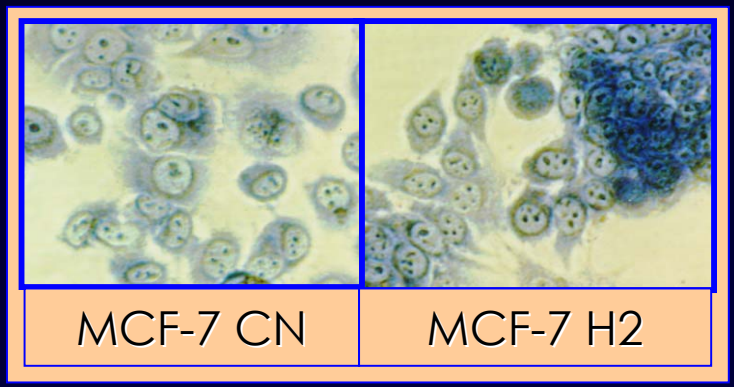


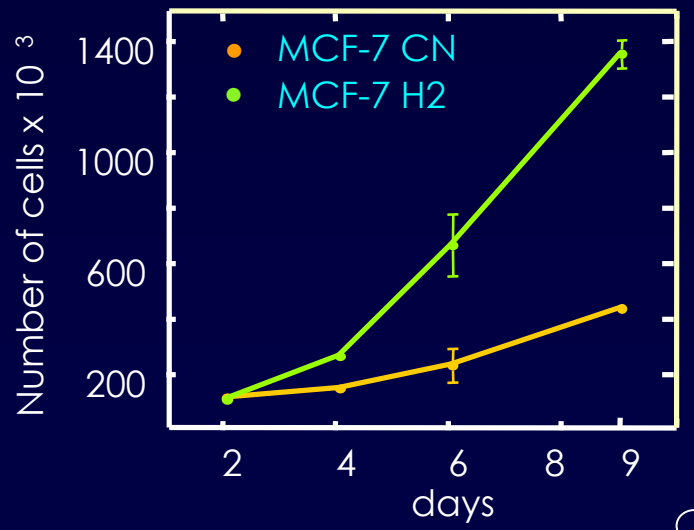
Engineered HER-2 Over-expression in MCF-7 cells
Increased Proliferation and Decreased Contact Inhibition

Anchorage-Independent Growth

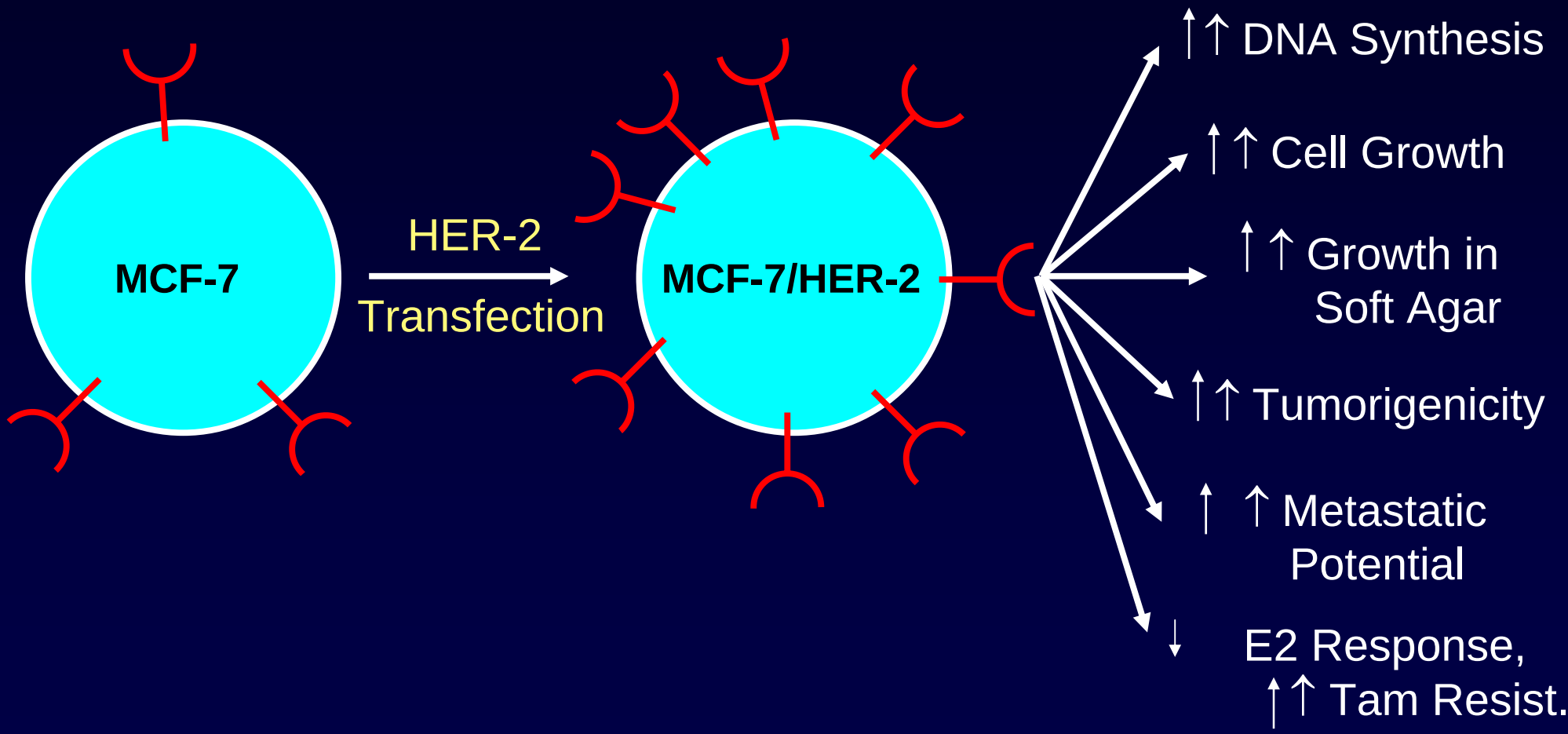


Growth on Plastic





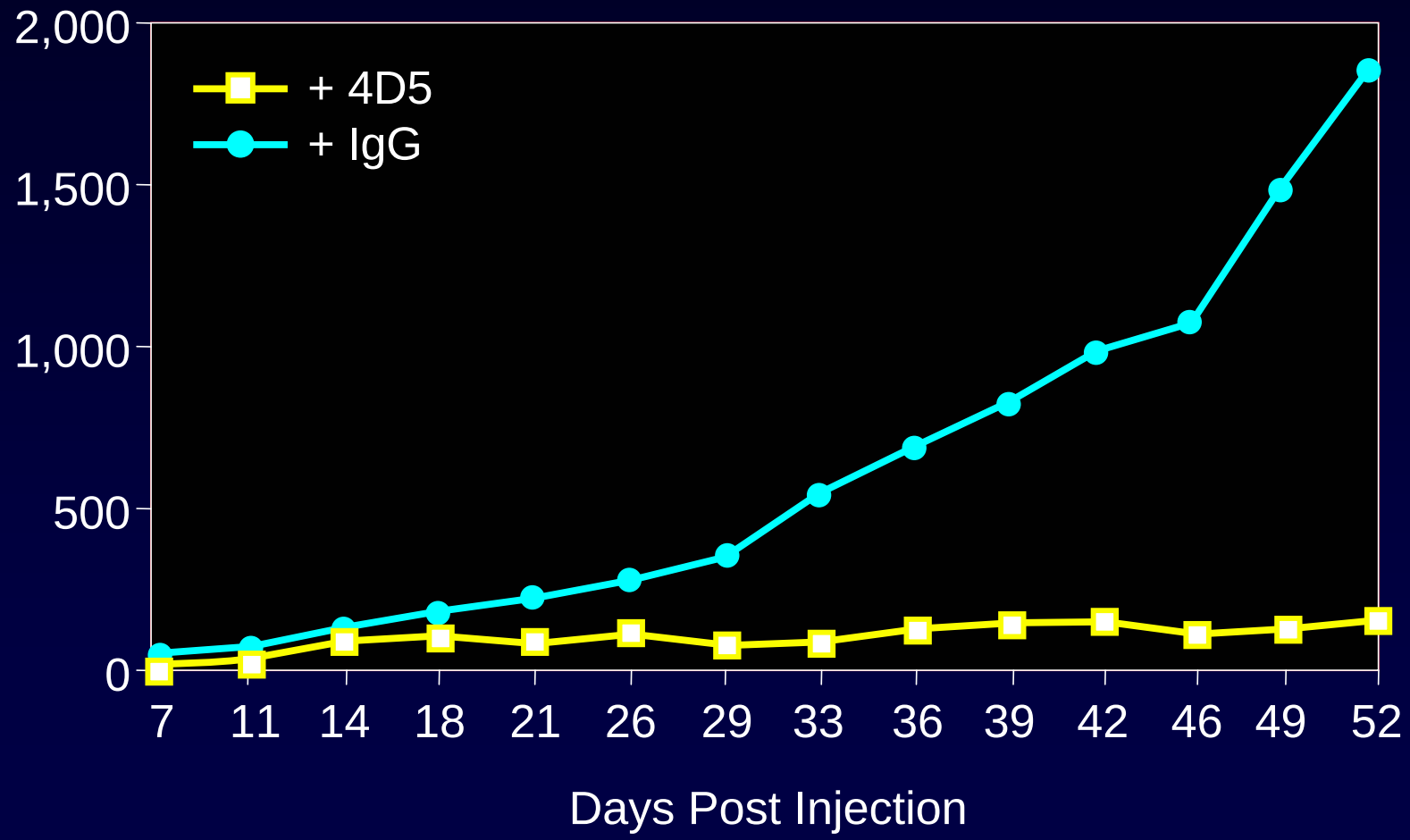
Biologic Effects of HER-2/*neu* Overexpression in Human Breast Cancer Cells



Target Validation

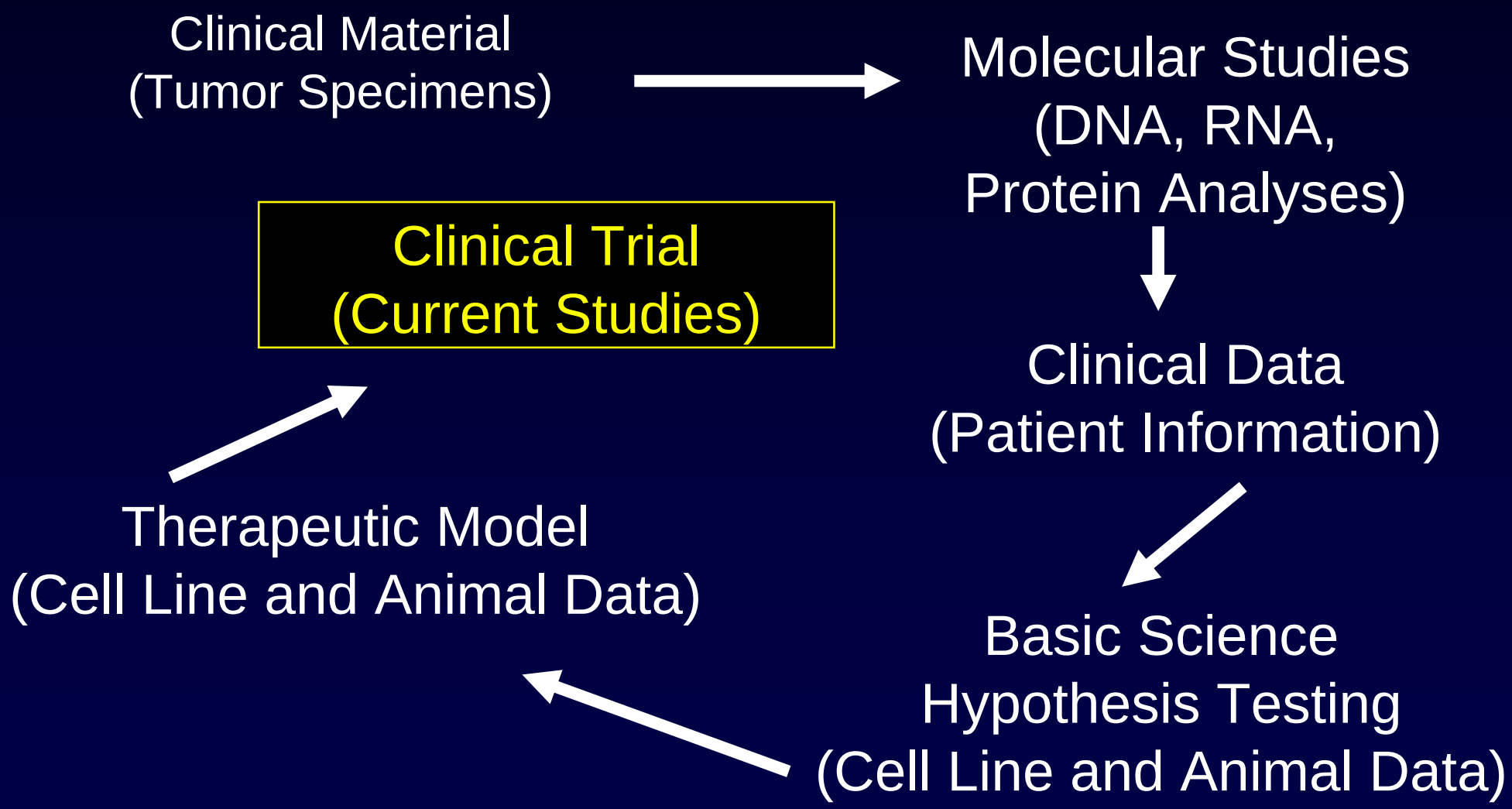
In Vivo Growth Inhibition Assay

MCF 7/HER-2



Clinical Translation

HER-2/neu Program at UCLA



Phase I Clinical Trials of Anti-HER-2 MAbs

Phase I	N	Study Design	Institution
MuMAb 4D5	20	Single dose (0.12 - 500 mg)	UCLA
H0453g	15	CDDP 100 mg/m^2 x 3 + rhuMAb HER-2 (10 - 500 mg x 9)	UCLA
H0452g	17	Multi-dose (10 - 500 mg)	UCLA, MSKCC, UCSF
H0407g	16	Single dose (10 - 500 mg)	UCLA, MSKCC

Herceptin in Combination with Chemotherapy

Objective - Combination Compared to Chemotherapy Alone

- Primary
  - Time to disease progression (REC)
  - Safety
- Secondary
  - Overall response rates
  - Durations of response
  - Time to treatment failure
  - 1-year survival
  - Quality of life

Herceptin in Combination with Chemotherapy

Design - Stratification to Chemotherapy

No prior anthracyclines → AC = doxorubicin (60 mg/m^2) or epirubicin (75 mg/m^2) + cyclophosphamide (600 mg/m^2) q 3 wks x 6 cycles

Prior anthracyclines → T = paclitaxel (175 mg/m^2 x 3 hr) q 3 wks x 6 cycles

Herceptin in Combination with Chemotherapy

Enrollment

Total enrolled	469	
Randomization	H + CT	CT
	235	234

Subgroups

H + AC	AC	H + T	T
143	138	92	96

Summary: Phase III Clinical Trial Comparing Best Available Chemotherapy to Same Therapy + Herceptin

	Enrolled	R.R. (%)	Dur. Res.	T.T.P
H + CT	235	49 (53%↑)	9.3M (58%↑)	7.6M (65%↑)
CT	234	32	5.9M	4.6M
H + AC	138	52 (20%↑)	9.1M (40%↑)	8.1M (33%↑)
AC	145	43	6.5M	6.1M
H + T	92	42 (163%↑)	11.0M (150%↑)	6.9M (130%↑)
T	96	16	4.4M	3.0M

Herceptin in Combination with Chemotherapy

Survival Time

- Overall Herceptin impact on survival uncertain
  - Limited duration of follow-up (≥12 months)
  - CT alone patients allowed to enter Herceptin extension protocol
- Preliminary analysis - improved 1-yr survival
  - H + CT = 78% alive
  - CT alone = 67% alive

Clinical Safety

Summary of Herceptin Safety

- Herceptin is generally well tolerated
  - Single agent
  - Combined with chemotherapy
- Most adverse events mild to moderate in severity
  - Infusion associated symptoms, including fever and chills primarily with first dose
- Serious adverse events infrequent
- Increased incidence of cardiac dysfunction, particularly when administered with anthracycline based therapy

Herceptin in Combination with Chemotherapy

Cardiac Dysfunction Outcomes (CREC)

	H + AC	AC	H + T	T
Cardiac Dysfunction Events (#)	**39 (27%)**	**9 (7%)**	**11 (12%)**	**2 (1%)**
Herceptin Rx Post Event (#)	**14**	**5***	**6**	**1***
Deaths (#)	**4**	**1**	**1**	**2**
MBC	**4**	**0**	**0**	**2**
Cardiac	**0**	**1**	**0**	**0**
Pneumonia	**0**	**0**	**1**	**0**

*Herceptin extension protocol

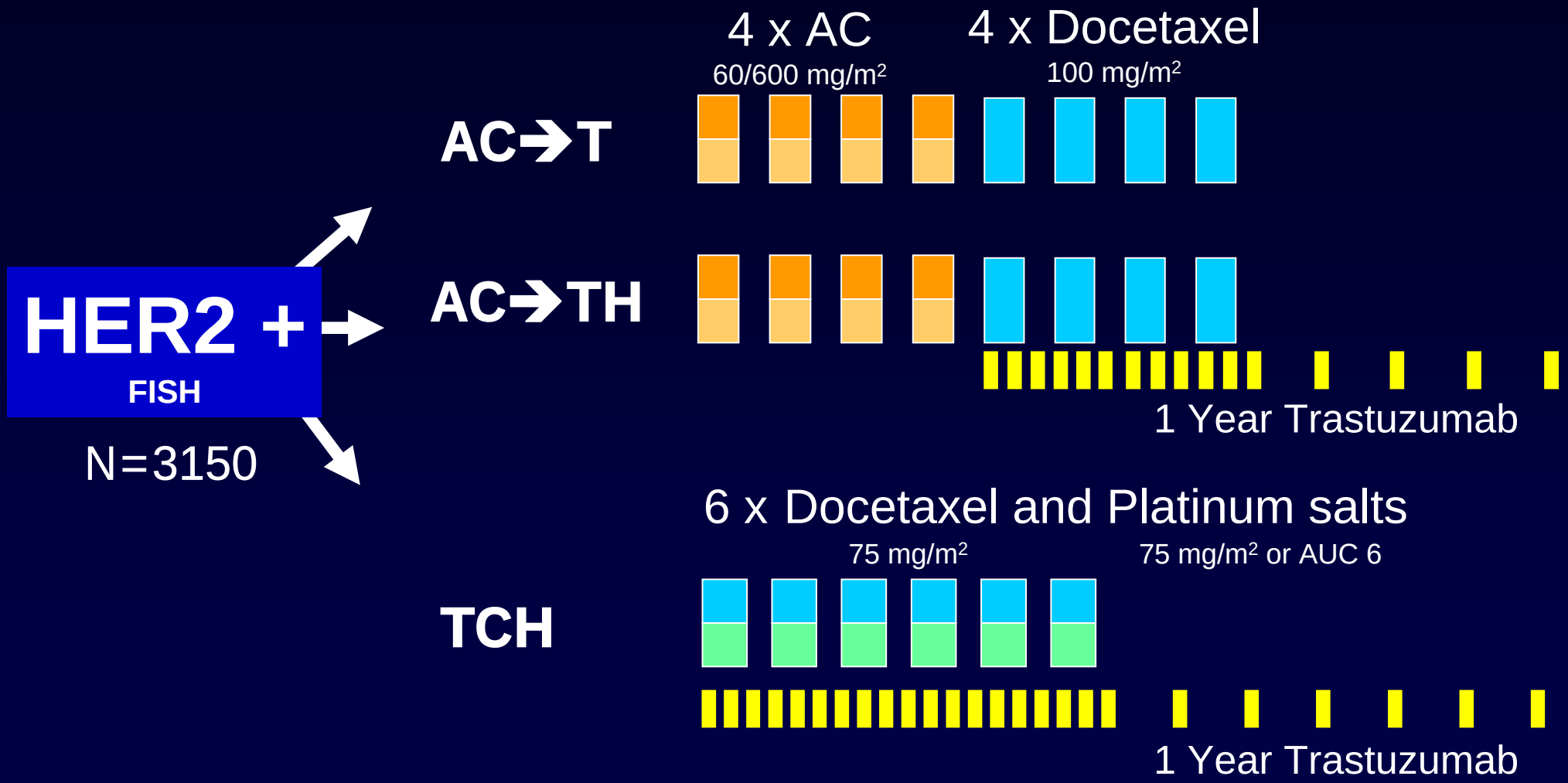
Conclusion

- The results of this study indicate that Herceptin™ (Trastuzumab) in combination with chemotherapy is well-tolerated and provides substantial clinical benefit in first-line treatment of HER-2 overexpressing metastatic breast cancer. Drug approved in Sept. 1998 as the first proto-oncogene kinase targeted therapeutic.
- Future studies of Herceptin will be important
  - Adjuvant breast cancer - preclinical data show earlier rx better
  - Other combinations

Adjuvant use of Herceptin must be evaluated in a randomized-controlled trial

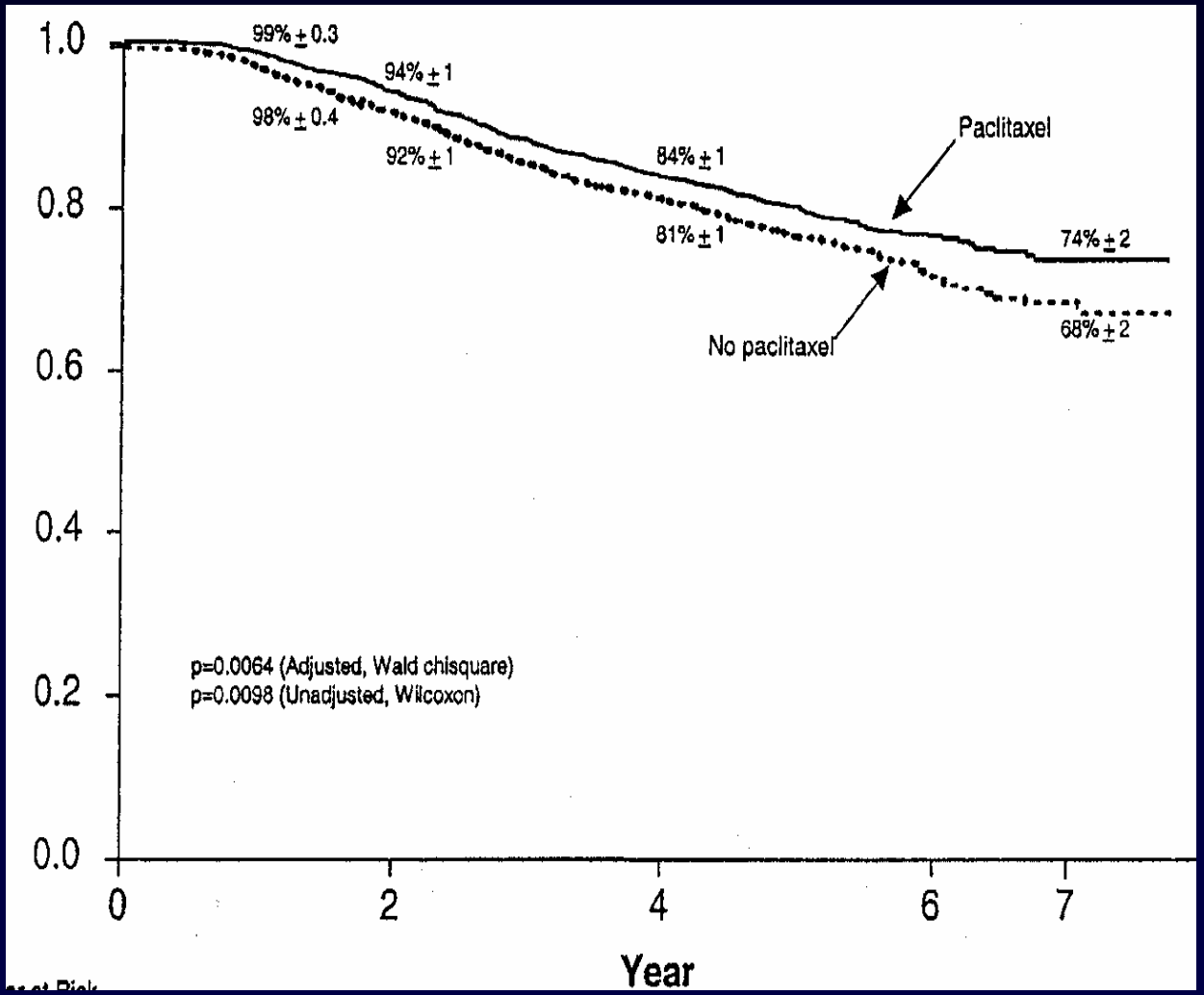
BCIRG 006

Adjuvant Breast Cancer
Node Positive and High Risk Node Negative



The “One-Size-Fits-All” Approach to Breast Cancer

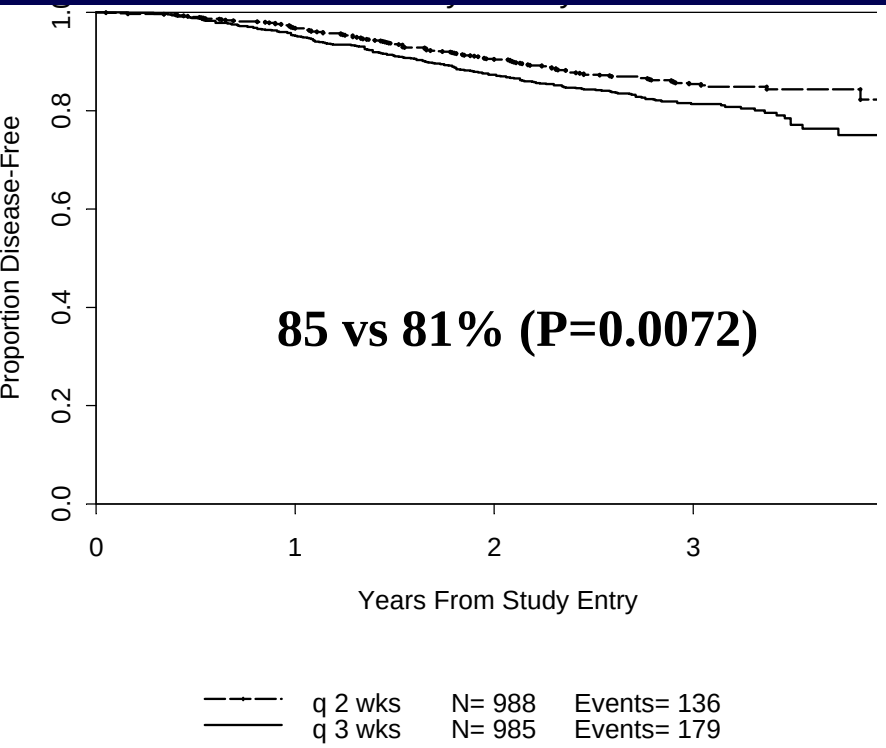
CALGB 9344: Overall Survival



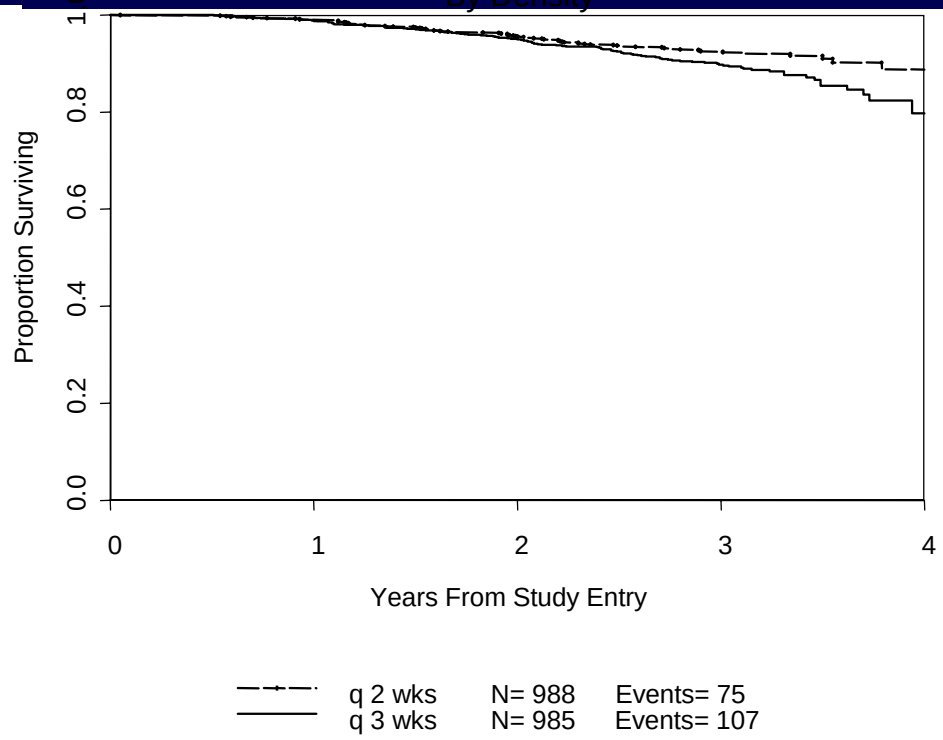
Henderson, et al. *J Clin Oncol.* 2003;21:976-83.

CALGB 9741
Interim Analyses

Disease-Free Survival

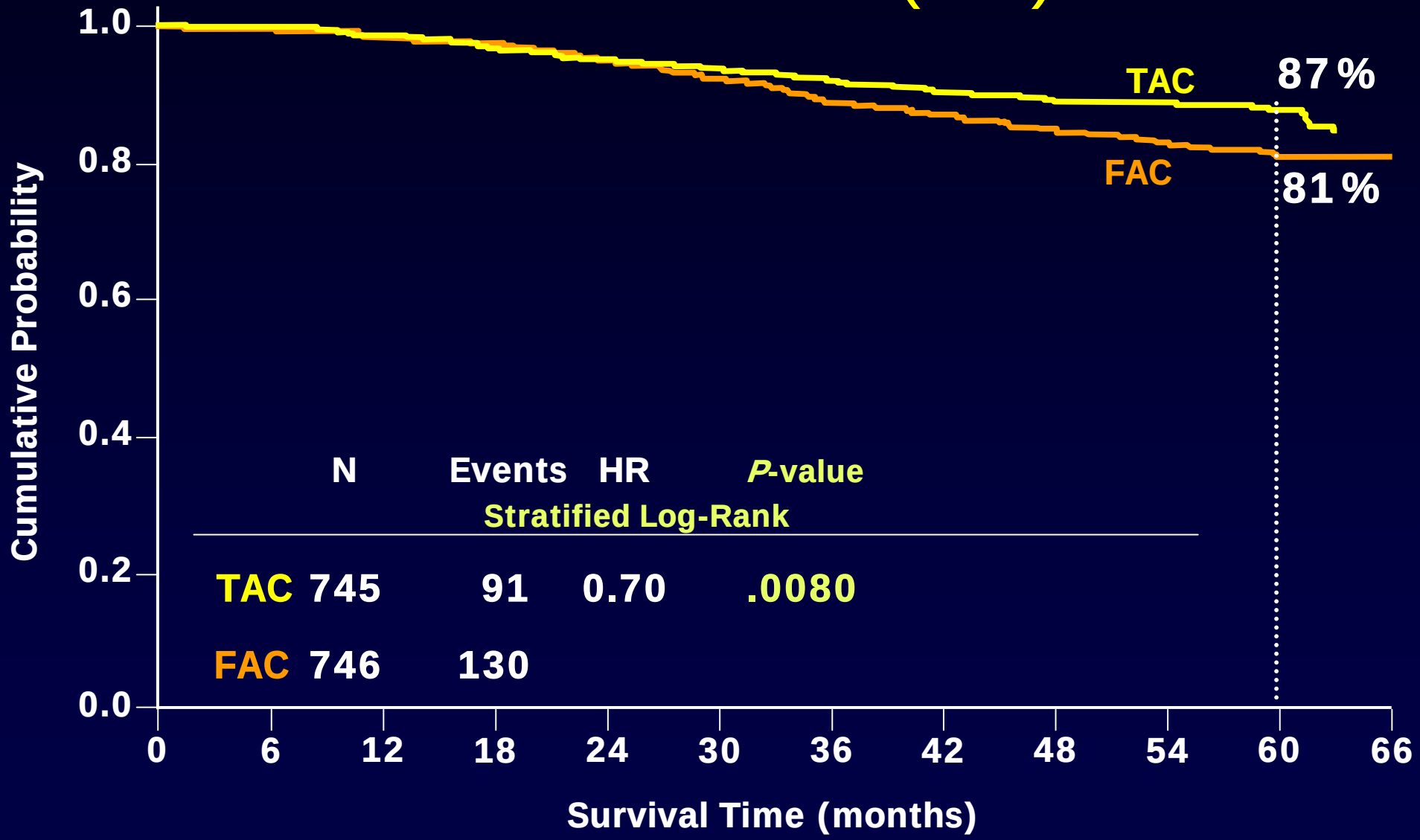


Overall Survival



N = 1973; Median F/U = 36 mos

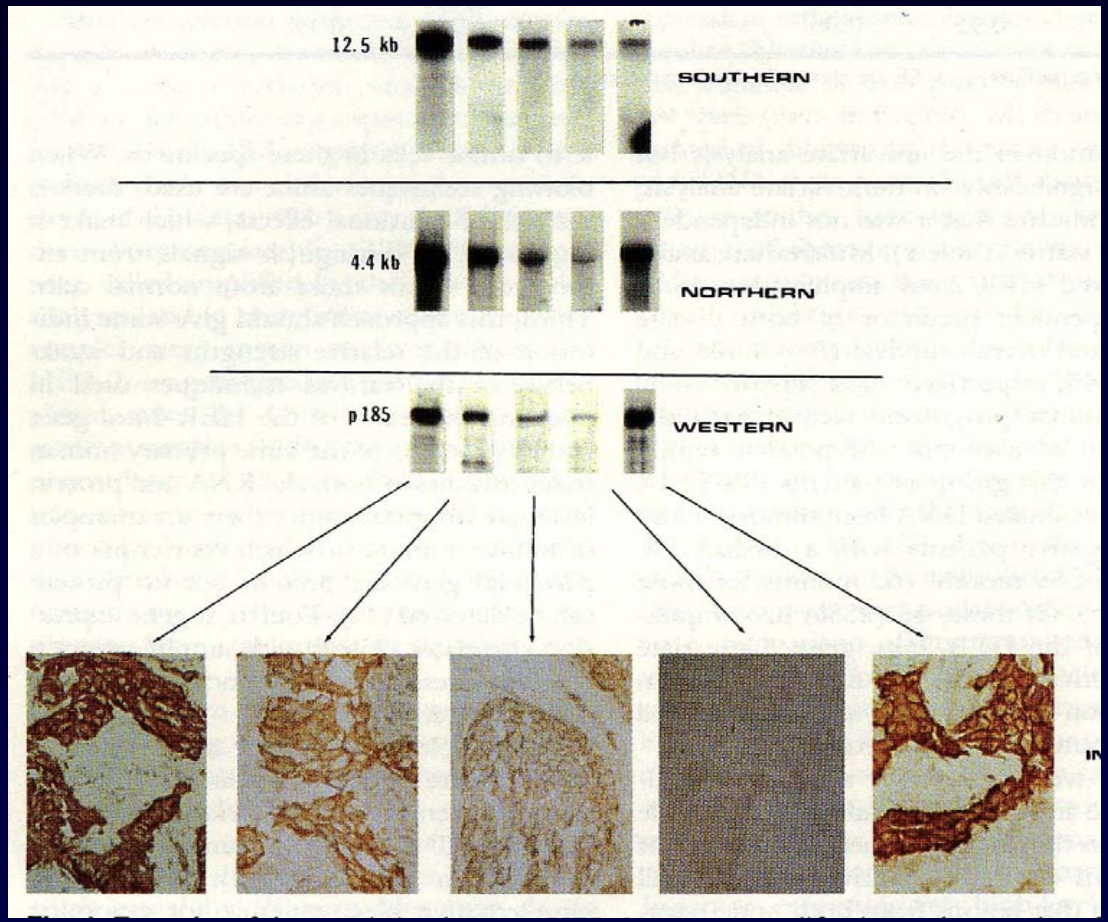
Overall Survival (ITT)



Can We Do Better?

The Hope - Clinical Translation of Biologically Relevant Molecular Information Should Lead to More Effective and Less Toxic Therapeutic Approaches

The HER2 Alteration



Southern

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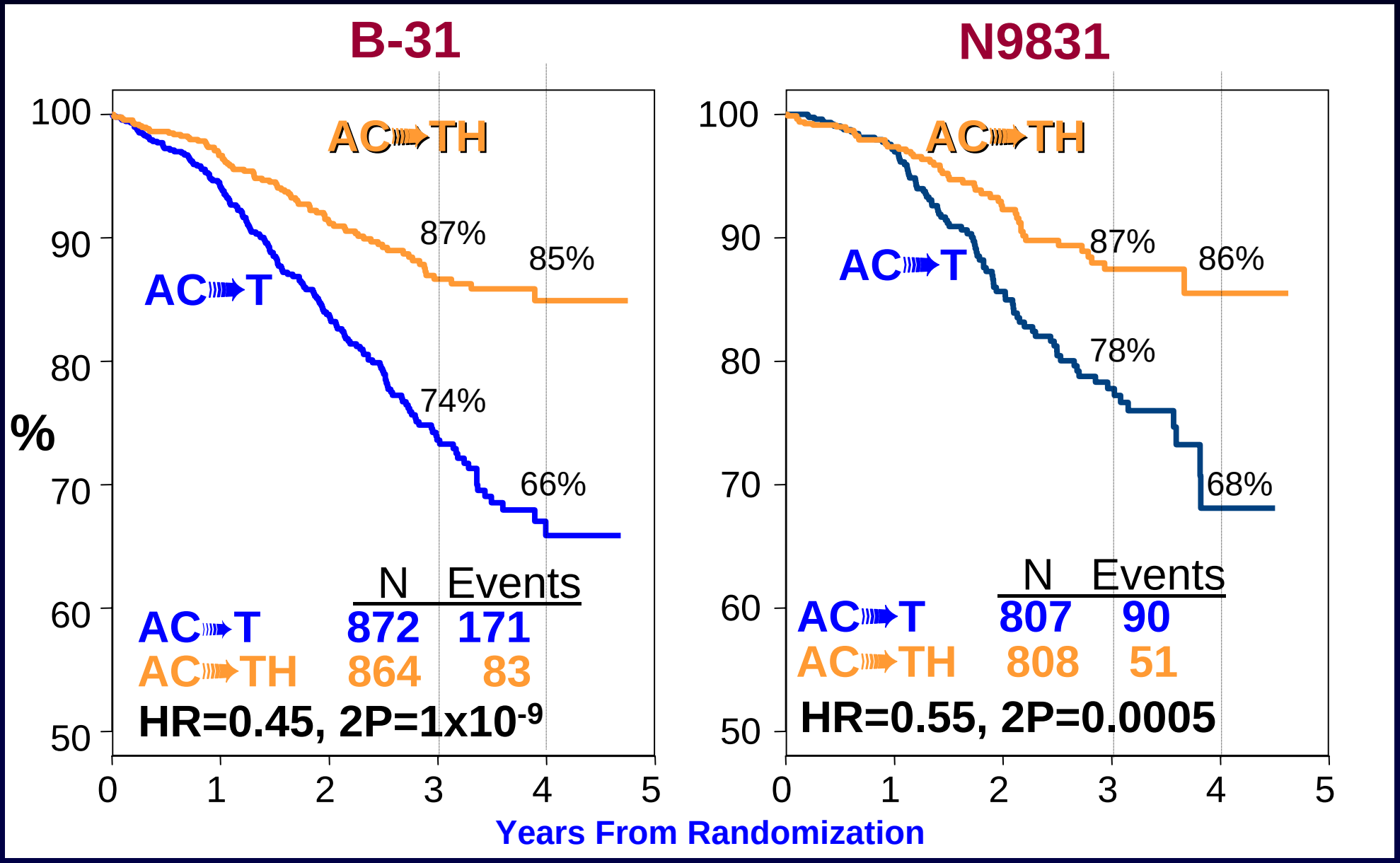
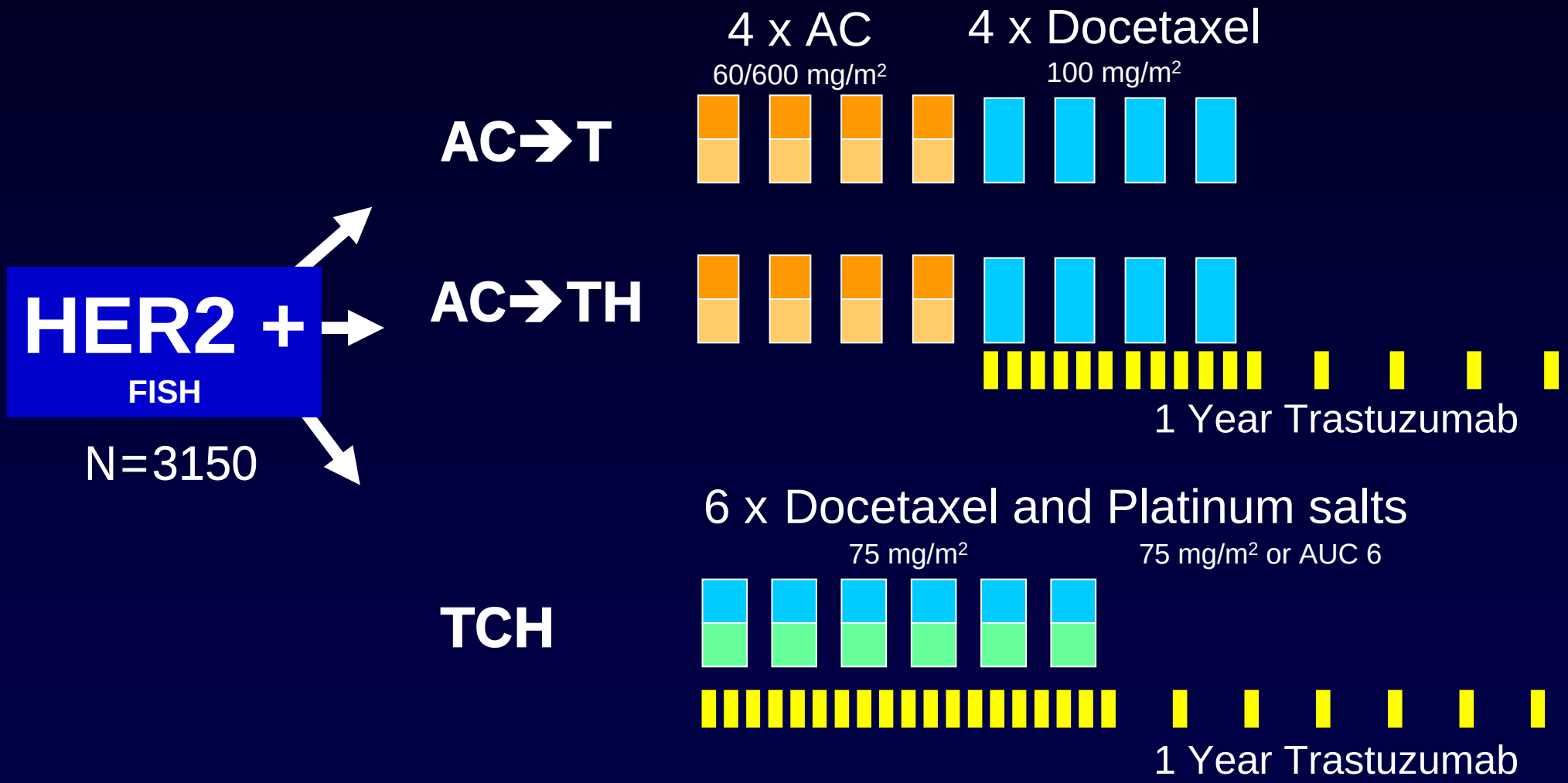
Slamon et al. *Science* 1989

Disease-Free Survival



BCIRG 006

Adjuvant Breast Cancer
Node Positive and High Risk Node Negative



LVEF Declines by NYHA Class

	AC-T	AC-TH	TCH
>10%, <LLN	9	34	7
>15%, <LLN	6	25	4
Grade 3/4 CHF	**2**	**20**	**1**

CIRG
Cancer International Research Group

THE FUTURE

Can We Do Even Better?

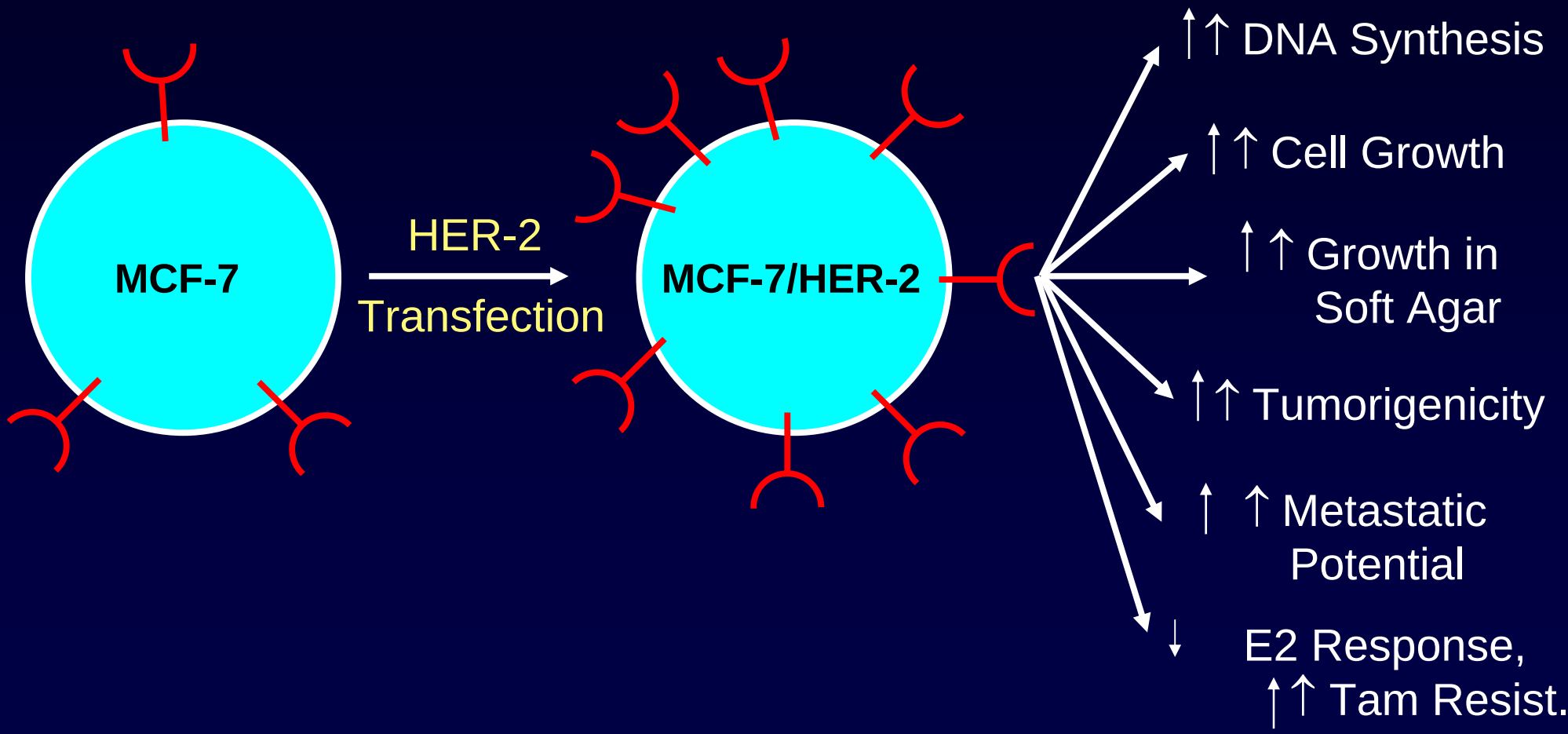
The Hope - Clinical Translation of Biologically Relevant Molecular Information Should Lead to Even More Effective and Less Toxic Therapeutic Approaches

Can we recognize molecular signaling pathway activation in cancer?

- Is activation ligand dependent?
- Are the initiating receptors interchangeable?
- What are the downstream effector genes?
- How do we determine if a cancer cell is dependent on a particular pathway or receptor?
- Can we identify gene signatures that predict response to molecularly targeted therapies?

Pathway Analysis

Biologic Effects of HER-2/*neu* Overexpression in Human Breast Cancer Cells



How Does an Alteration in This One Gene Result in So Many Changes in Biologic Behavior?

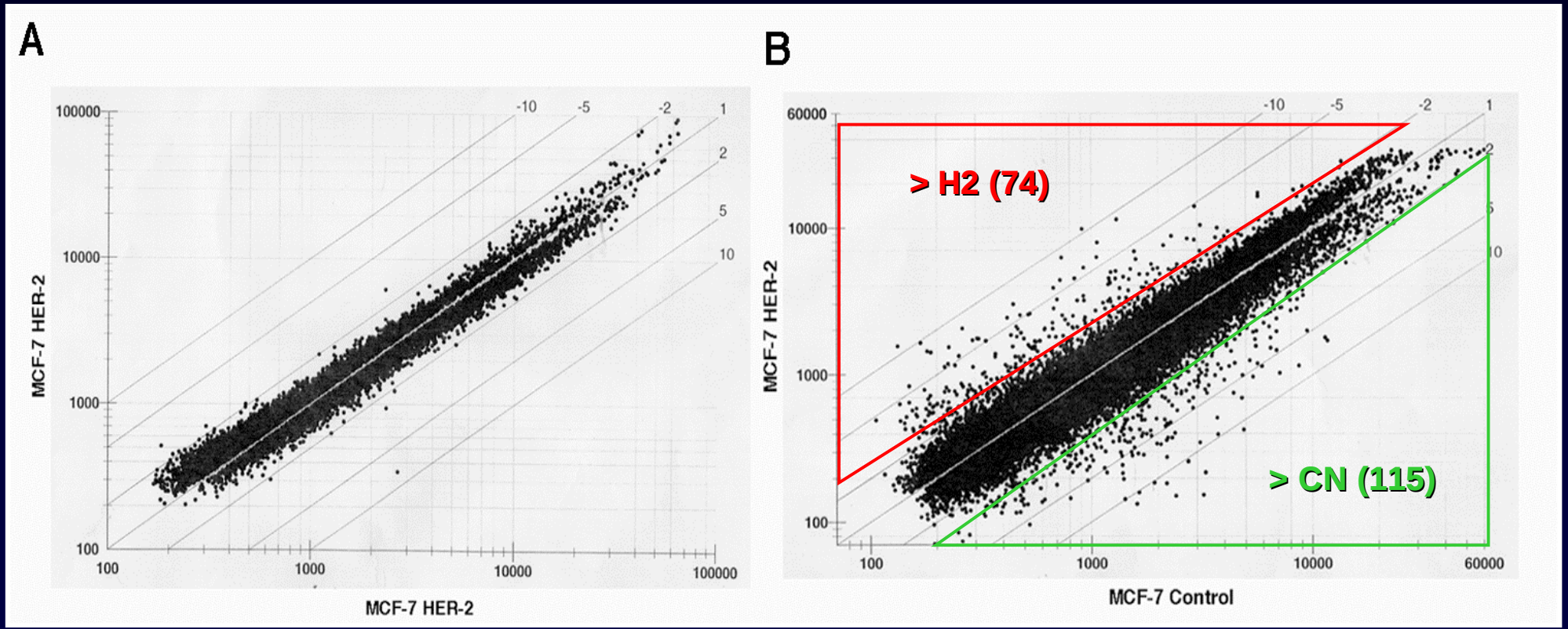
- While it is an important “inciting” event, amplification of HER2/neu does not cause it’s associated clinical phenotype in isolation.
- What other genes and/or pathways need to be engaged to bring about this profound clinical picture?
- A better understanding of those genes and/or pathways directly associated with the HER2/neu alteration will lead to more effective therapeutic approaches

- **Global gene expression profiling**
- Confirmation of expression
- Possible Biologic Relevance
- Confirmation of Functional Relevance

cDNA Microarrays

Synteni/Incyte Double Fluorescence Method

GEMS 1-4, V (representing 40,000 elements)



Self RNA test

MCF-7/H2 v.s. CN

490 elements Δ > 2.5 fold

Data Analysis

- Clustering:
  - gene expression relatedness
- Pathway construction:
  - biologically biased hierarchical ordering

Summary: cDNA Microarray

	[a]MCF-7 HER-2 down	[b]MCF-7 HER-2 up
receptors	12	8
growth factors, cytokines	8	5
GF induced proteins	10	0
cell cycle related	1	11
apolipoprotein related	8	0
cell adhesion-cytoskeleton	26	31
oncogenes/transcription factors	19	7
proteases and protease inhibitors	3	5
DNA/chromosome maintenance	5	2
drug resistance	0	10
complimen t related	1	3
housekeeping/chaperone proteins	10	3
nucleotide exchange factors	3	1
tRNA synthetases	0	8
enzymes/metabolism	20	12
misc. surface antigens	0	0
uncatagorized known genes	29	13
unknown genes	20	7
EST with homology	24	15
EST without homology	103	47
total changes greater than 2.5 fold	**302**	**188**

Selection Criteria for Analysis of Differentially Expressed Genes

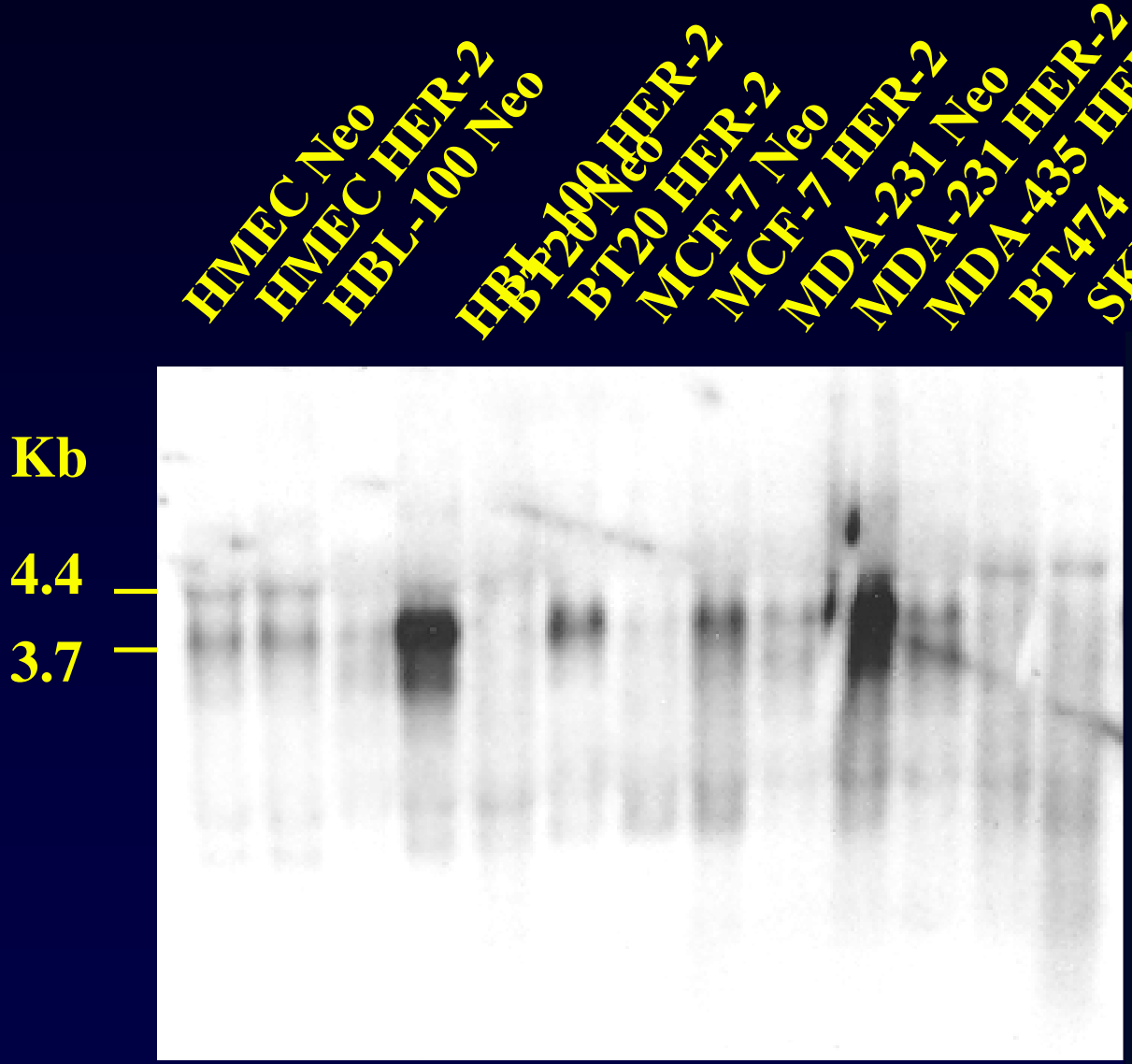
- Genes falling into identifiable pathways
- Genes effected in multiple cell lines
- Changes most likely to directly contribute to the HER-2/*neu* phenotype
- Expression changes reversed by Herceptin

Angiogenic Pathways

Gene name	MCF-7 con vs H2	ZR-75 con vs H2	LnCap con vs H2	SKBR3 W/Hcpt
VEGF	1.64 (f)	4.5 (f) 2.7 (c)	2.2 (f)	-
Angiopoietin-1	4.2 (f)	-	-	1.9 (f)
FGFR 4	2.8 (f)	2.3 (f)	-	-

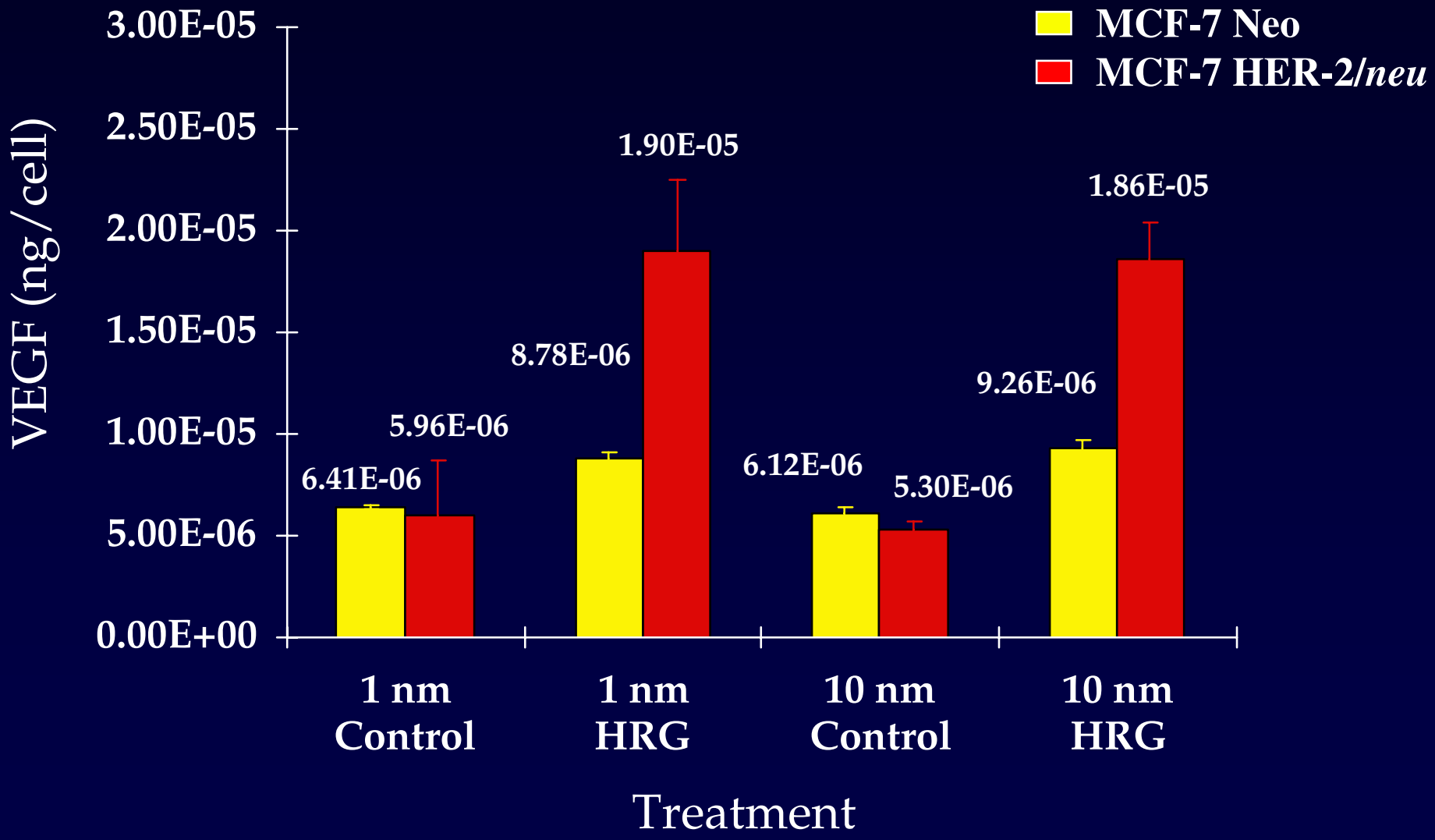
- Global gene expression profiling
- **Confirmation of expression**
- Possible Biologic Relevance
- Confirmation of Functional Relevance

Cell Line RNA Northern: VEGF Probe



Does activation of HER-2/*neu* result in increased VEGF production?

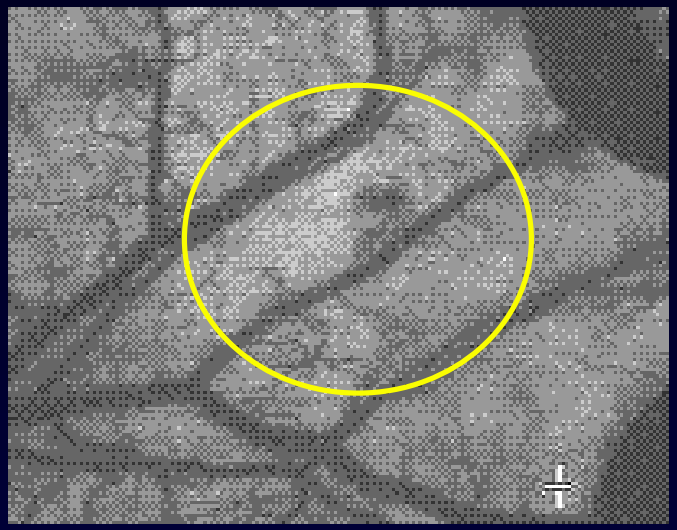
Concentration of VEGF in Conditioned Media of MCF-7 Neo and MCF-7 HER-2/*neu* Cells

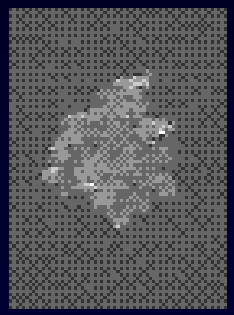


- Global gene expression profiling
- **Confirmation of expression**
- Possible Biologic Relevance
- Confirmation of Functional Relevance

Are the increased VEGF levels in HER-2/*neu* transfectants associated with increased angiogenesis *in vivo*?

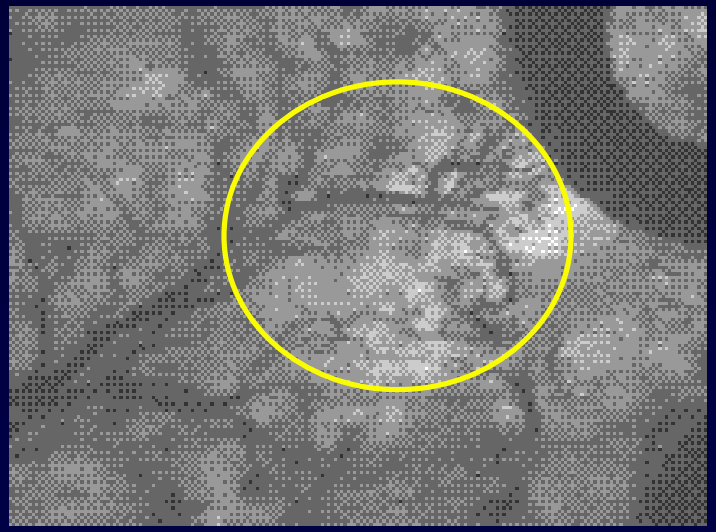
Angiogenesis in MCF-7 Spheroids: Day 0

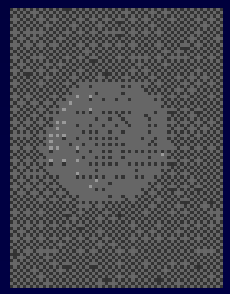




MCF-7 Neo:

1 x mag.
913 µm x 789 µm

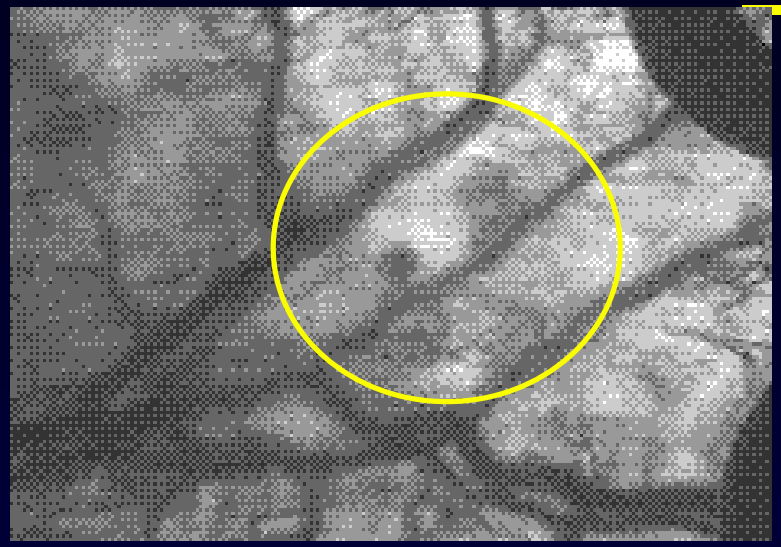




MCF-7 HER-2/*neu*:

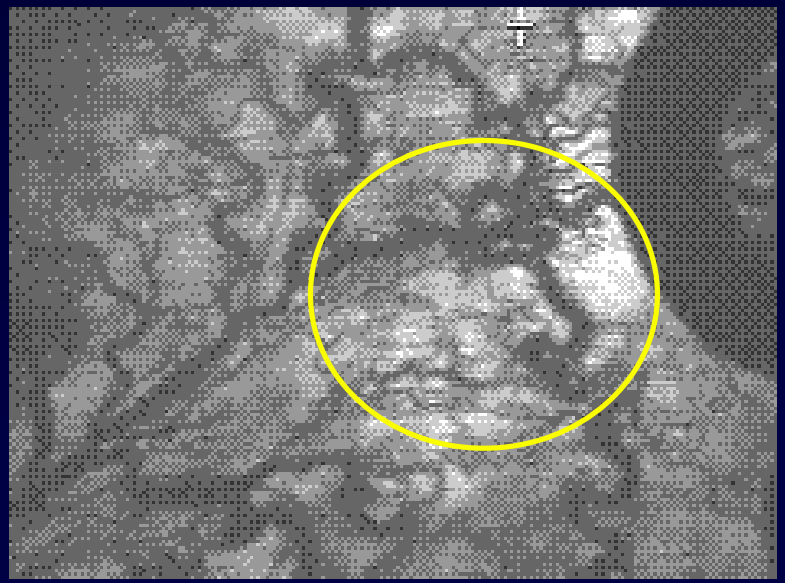
1 x mag.
876 µm x 857 µm

Angiogenesis in MCF-7 Spheroids: Day 3



MCF-7 Neo:

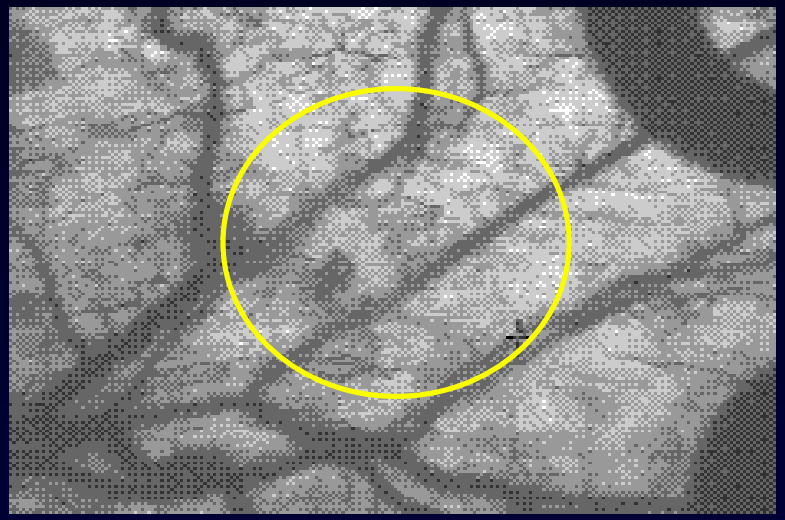
1 x mag.
-Vessel buds starting to form
-Vessels dilated



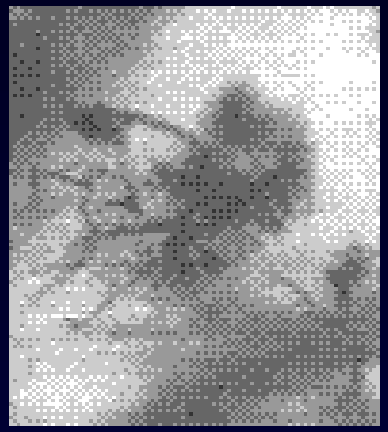
MCF-7 HER-2/*neu*:

1 x mag.
-Increased # of vessels
- Vessels dilated
- Vessels tortuous

Angiogenesis in MCF-7 Spheroids: Day 7



1 x



10 x

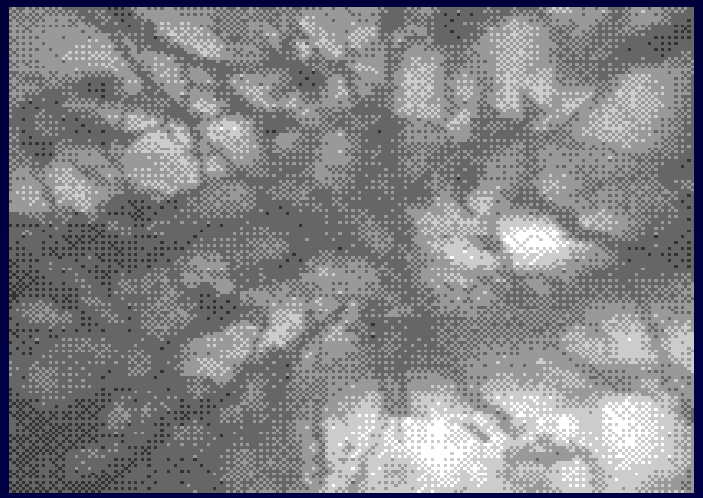
MCF-7 Neo:

1 x mag.

- Small capillaries and a few buds present

10 x mag.

- Vessels hemorrhaging

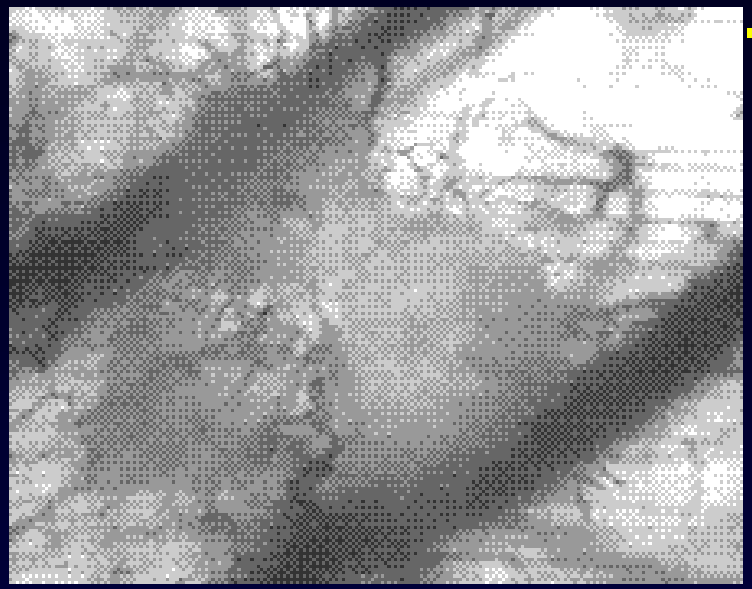


MCF-7 HER-2/*neu*:

3.5 x mag.

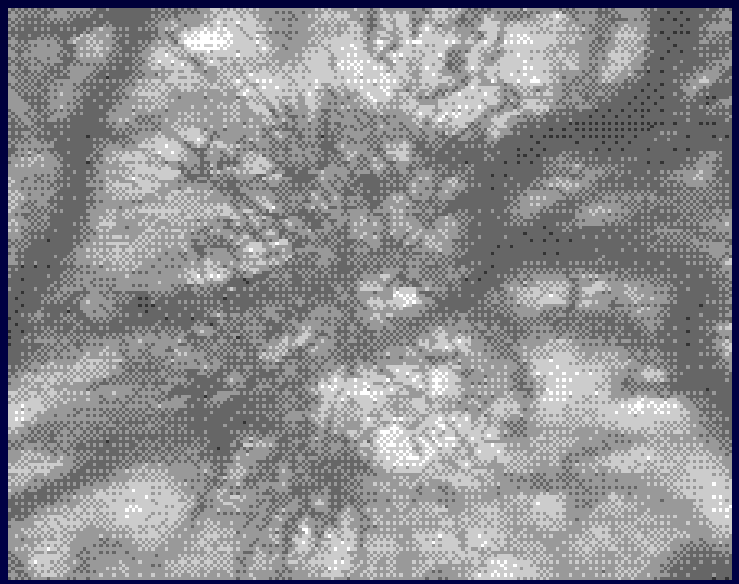
- Huge vessel network
- Large amount of vessel budding

Angiogenesis in MCF-7 Spheroids: Day 14



MCF-7 Neo:

3.5 x mag.
-Mature vasculature
- No vessel buds
-Development stopped



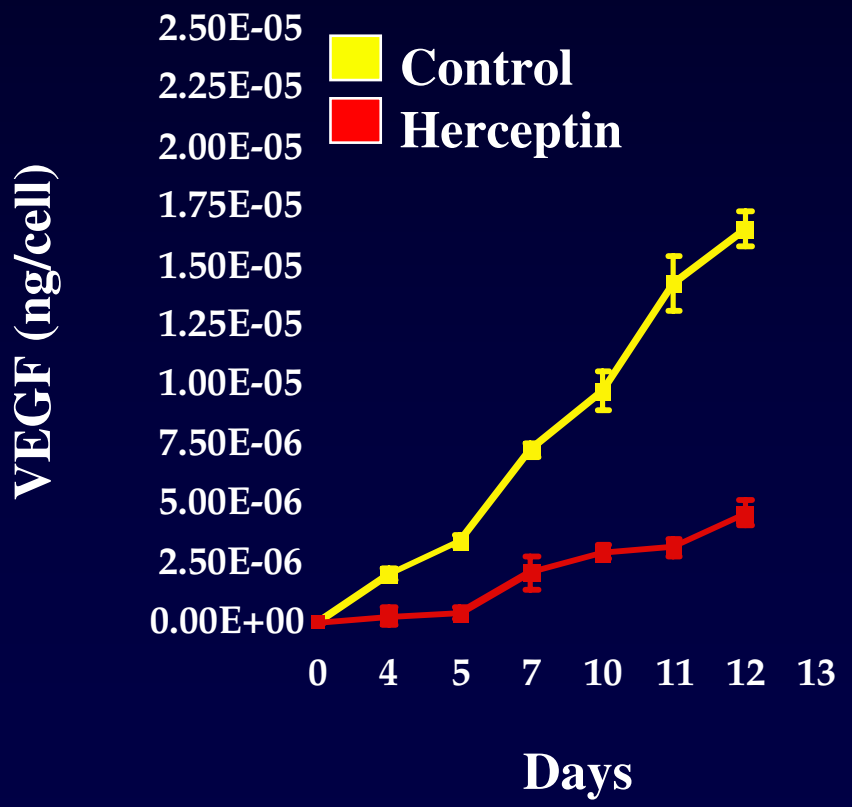
MCF-7 HER-2/*neu*:

10 x mag.
-High number mature vessels
- Vessel buds in center of tumor
- Vasculature still growing

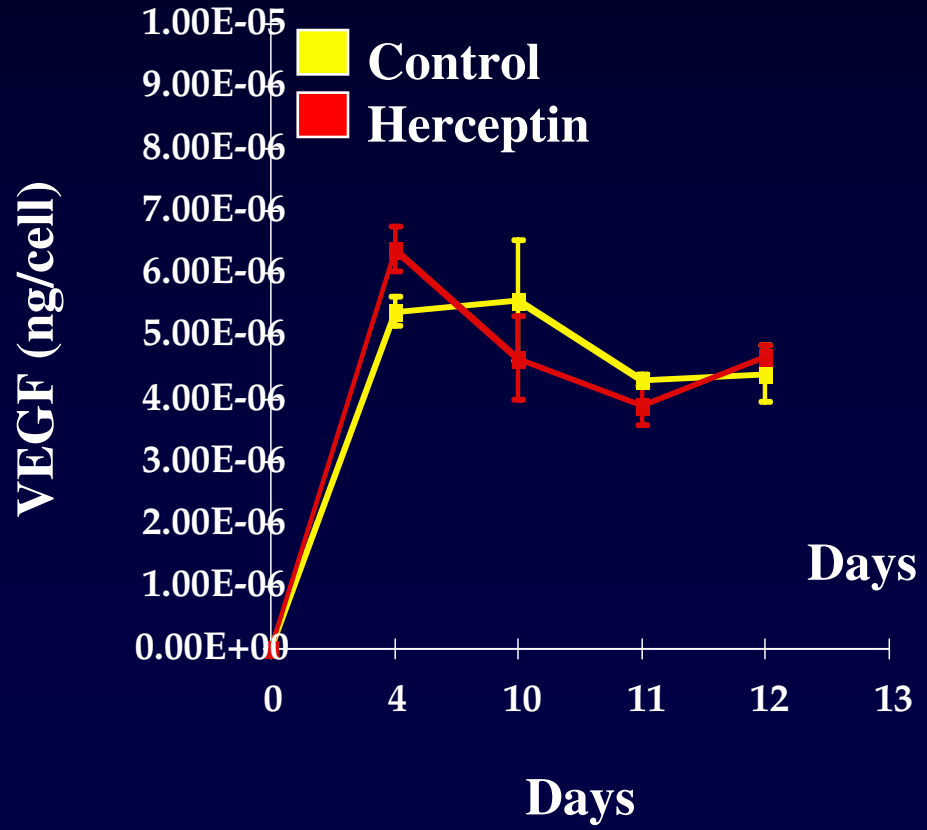
Does Herceptin decrease the levels of VEGF production in tumor cells?

Levels of VEGF in MCF-7 Cells after Herceptin Treatment

MCF-7 HER-2/*neu* Cells



MCF-7 Neo Cells

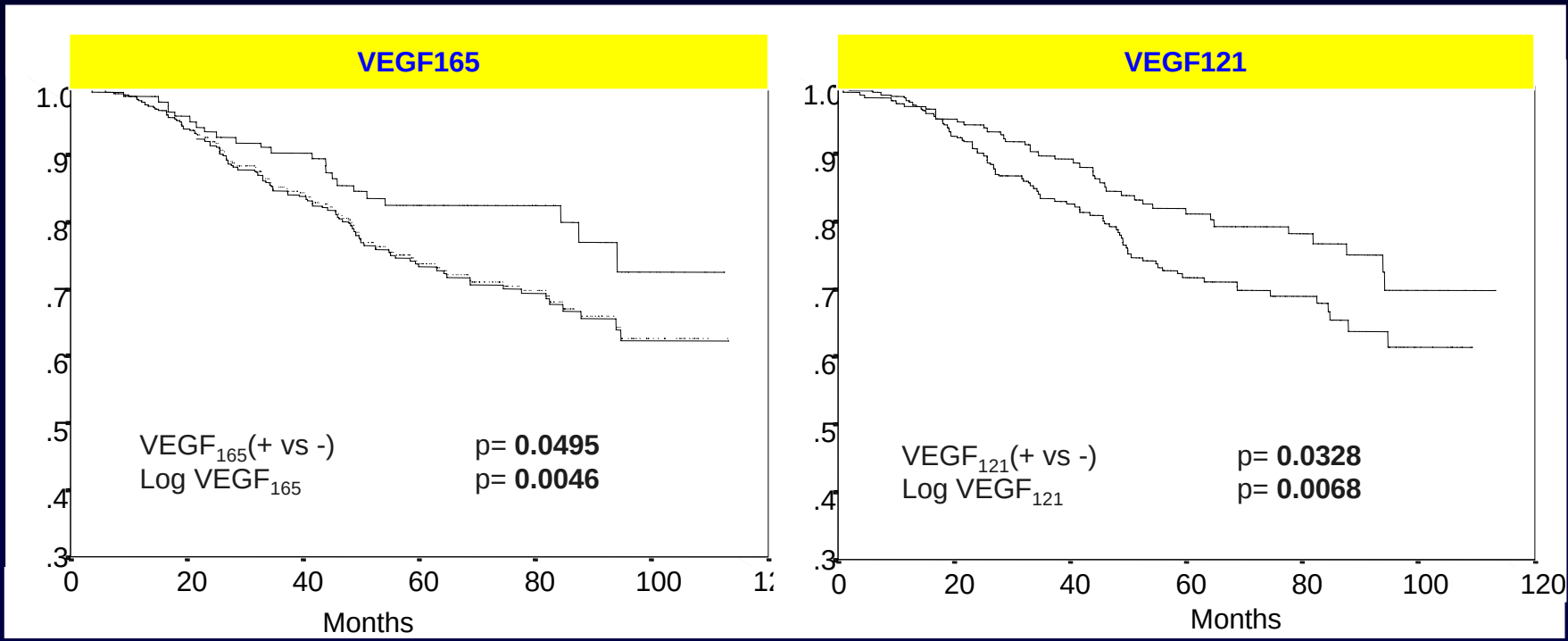


Do the Preclinical Data Translate to Findings in Clinical Specimens?

Patient and disease characteristics in node-negative and -positive primary breast cancer patients (n=611)

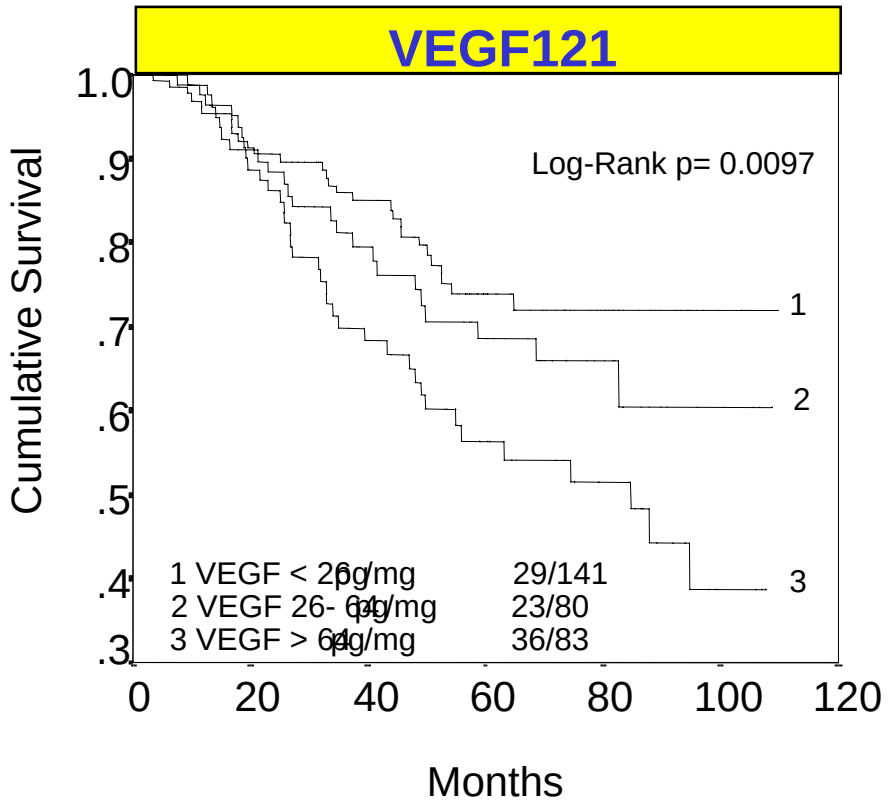
Factors		Number of Patients	%
Age		58 years	611
Tumor size*			
	(<2 cm)	231	38.2
	(2-4.9 cm)	310	51.2
	(≥5 cm)	64	10.6
Number of positive nodes*			
	0	290	48.7
	1-3	183	30.7
	4-9	61	10.3
	≥10	61	10.3
Lymph node status			
	Negative	290	48.3
	Positive	310	51.7
Nuclear grade*			
	1-2	368	60.4
	3-4	241	39.6
Hormone receptor status**			
	Negative	137	22.4
	Positive	474	77.6
HER-2/neu status***			
	Negative	497	81.3
	Positive	114	18.7
$VEGF_{121}$ status****			
	Negative	252	41.2
	Positive	359	58.8
$VEGF_{165}$ status****			
	Negative	158	25.9
	Positive	453	74.1

Prognostic Significance of Detectable $VEGF_{165}$ and $VEGF_{121}$ Expression for Survival in Primary Breast Cancer



Konecny G, et al.: Clin Cancer Res in press, 2004

A biological concentration-effect relationship between VEGF expression and survival



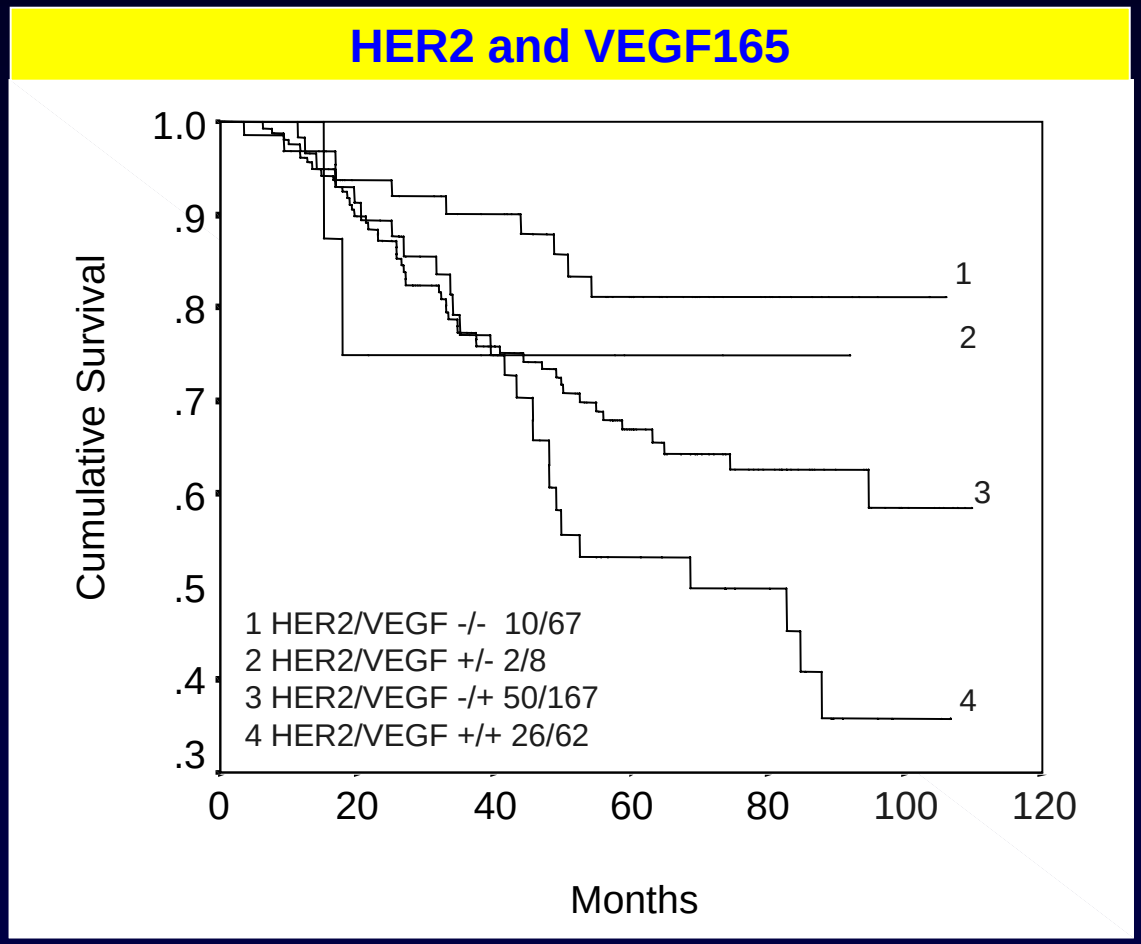
Correlation between HER2 and $VEGF_{121}$ in Primary Breast Cancer

	$VEGF_{121}$		
	negative	positive*	Total
HER2 negative	226 (45.5%)	271 (54.5%)	480 (100%)
HER2 positive	26 (22.8%)	88 (77.2%)	108 (100%)

Chi-Square Test: $p < 0.001$
*** $VEGF_{121}$-positive - detectable $VEGF_{121}$ levels above the lower assay sensitivity of 16 pg/ml**

Konecny G, et al.: Clin. Cancer Res, 2004, 10:1706-1716

Combined effects of HER2 and $VEGF_{165}$ expression on survival

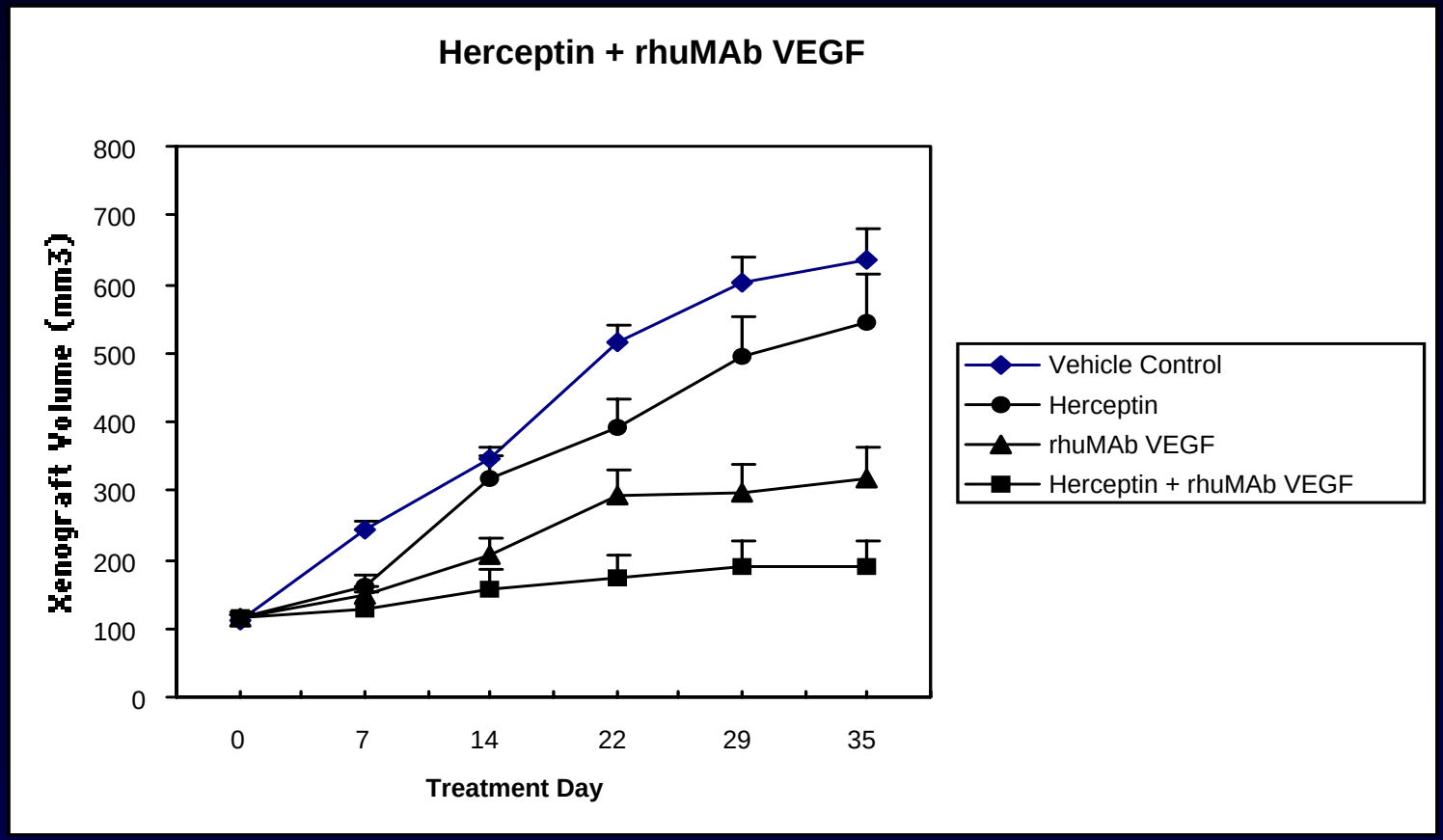


Konecny G, et al.: Clin. Cancer Res. 2004, 10:1706-1716

- Global gene expression profiling
- Confirmation of expression
- Possible Biologic Relevance
- **Confirmation of Functional Relevance**

What is the effect of Herceptin and the VEGF antibody on tumor growth *in vivo*?

Effect of Herceptin, rhuMAb VEGF, and the Combination against HER2-overexpressing xenografts.



Pegram, et al., Phase I/II Investigator-initiated Trial "Combined biologic therapy of breast cancer", DAMD BC004021, (2001)

Do the Preclinical Therapeutic Data Translate into the Clinic?

Phase I/II clinical trial of Herceptin and Avastin in breast cancer

Hypothesis: upregulation of VEGF in HER2+ MBC contributes to the aggressive phenotype of HER2+ MBC. The 'angiogenic switch' modulated by Herceptin can be exploited in the clinic by combined blockade of these two "linked" pathways

LABC or MBC
HER2+ by FISH
ECOG 0-1
Age >18 Y
LVEF WNL

Herceptin 4mg/kg → 2mg/kg qw

Avastin dose escalation (n=24)

A 3mg/kg → 5mg/kg →10mg/kg
IV d7 then q14d

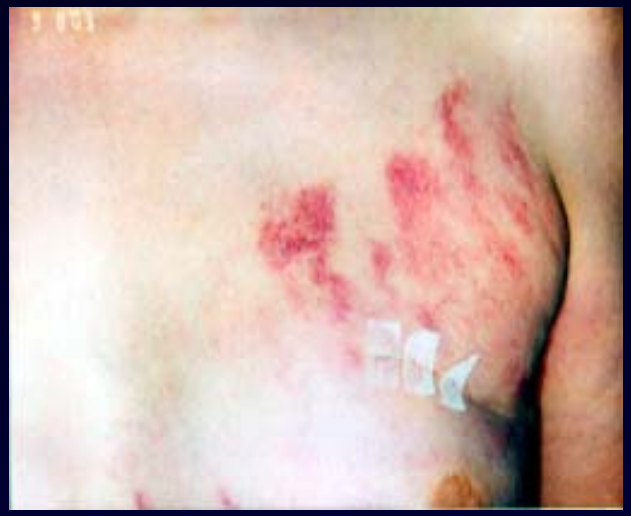
↓

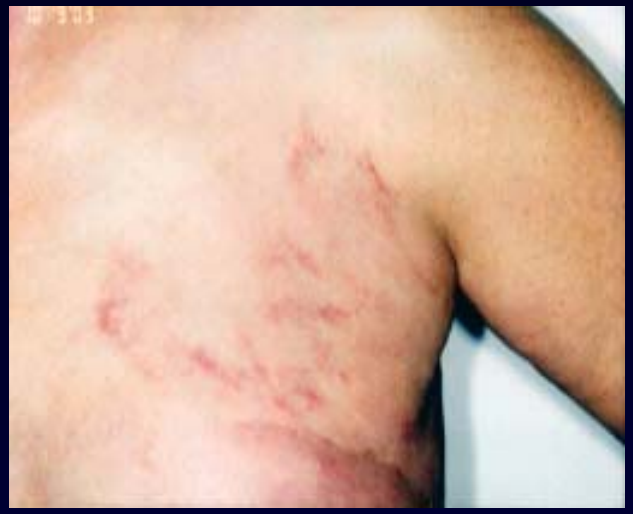
Herceptin 4mg/kg → 2mg/kg qw
+
Avastin q14d

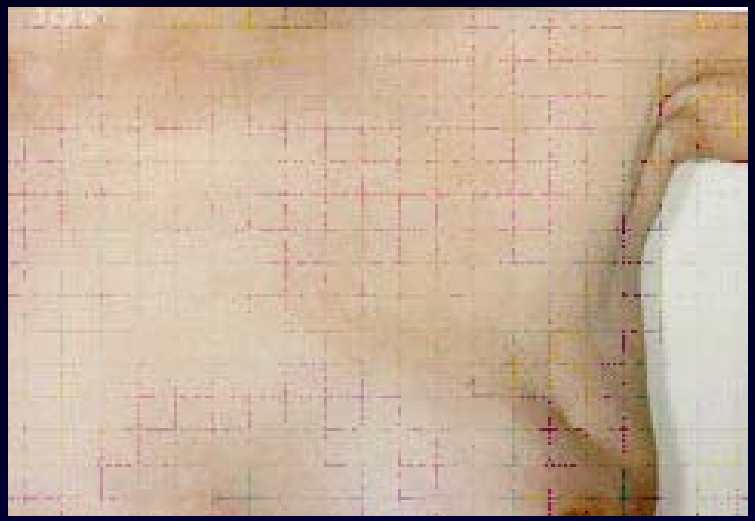
Study Endpoints

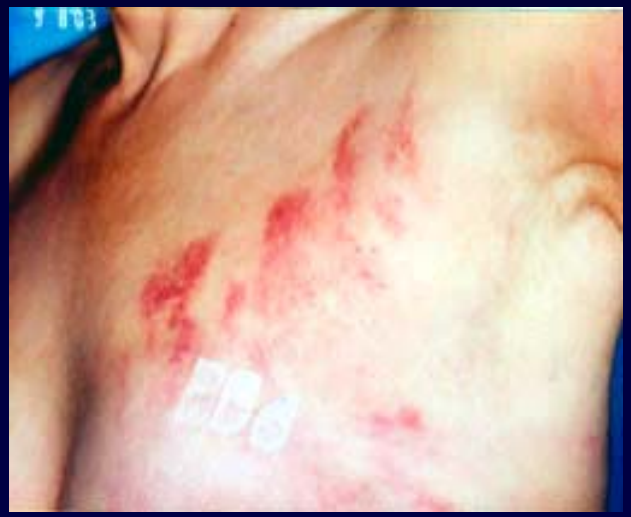
1. **Clinical Safety**
2. **Pharmacokinetics**
3. **Efficacy**

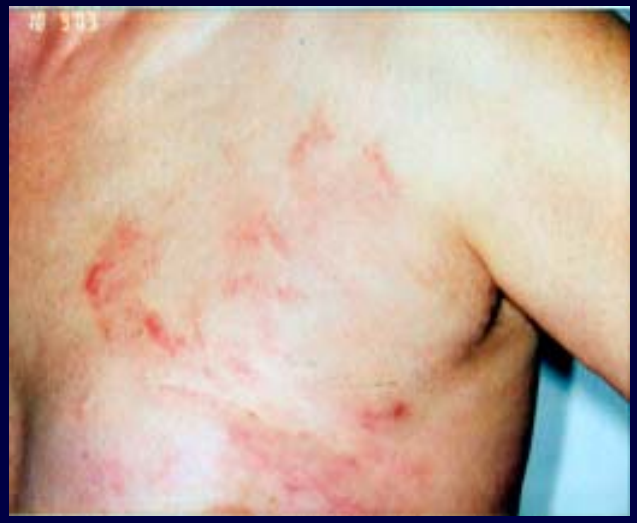
Day 0 1 month 9 months







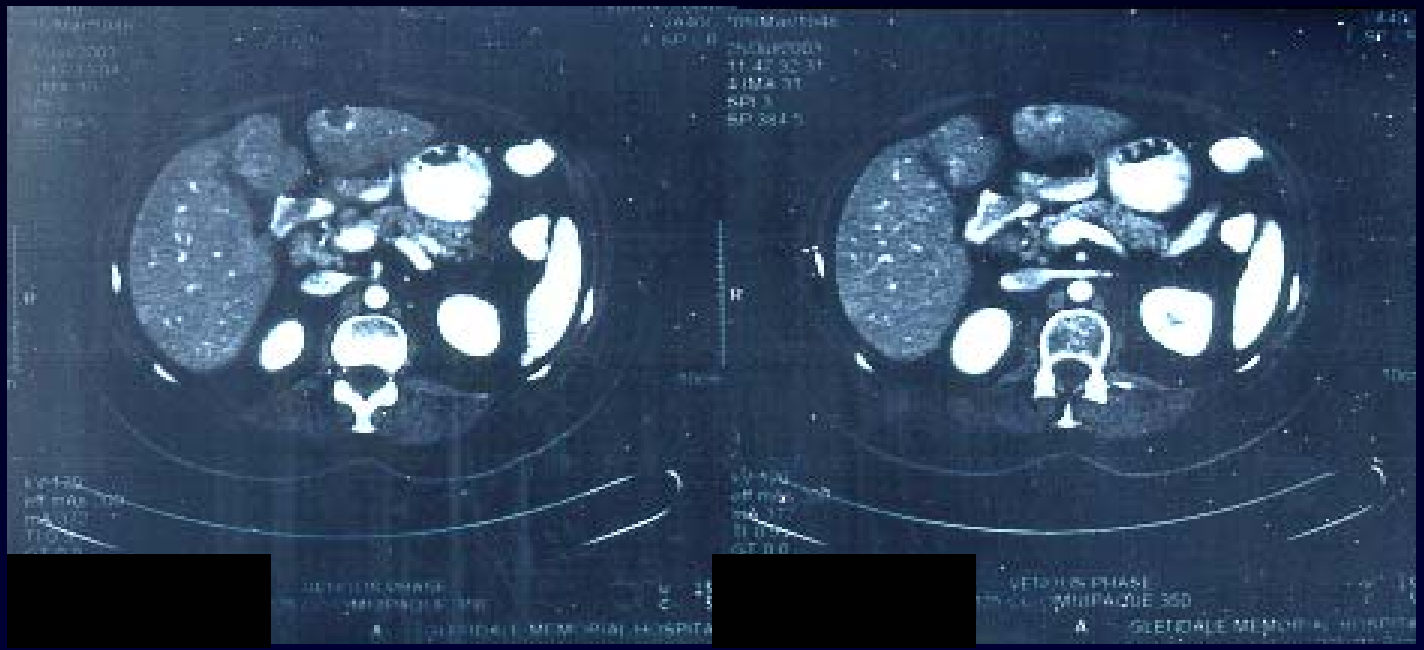


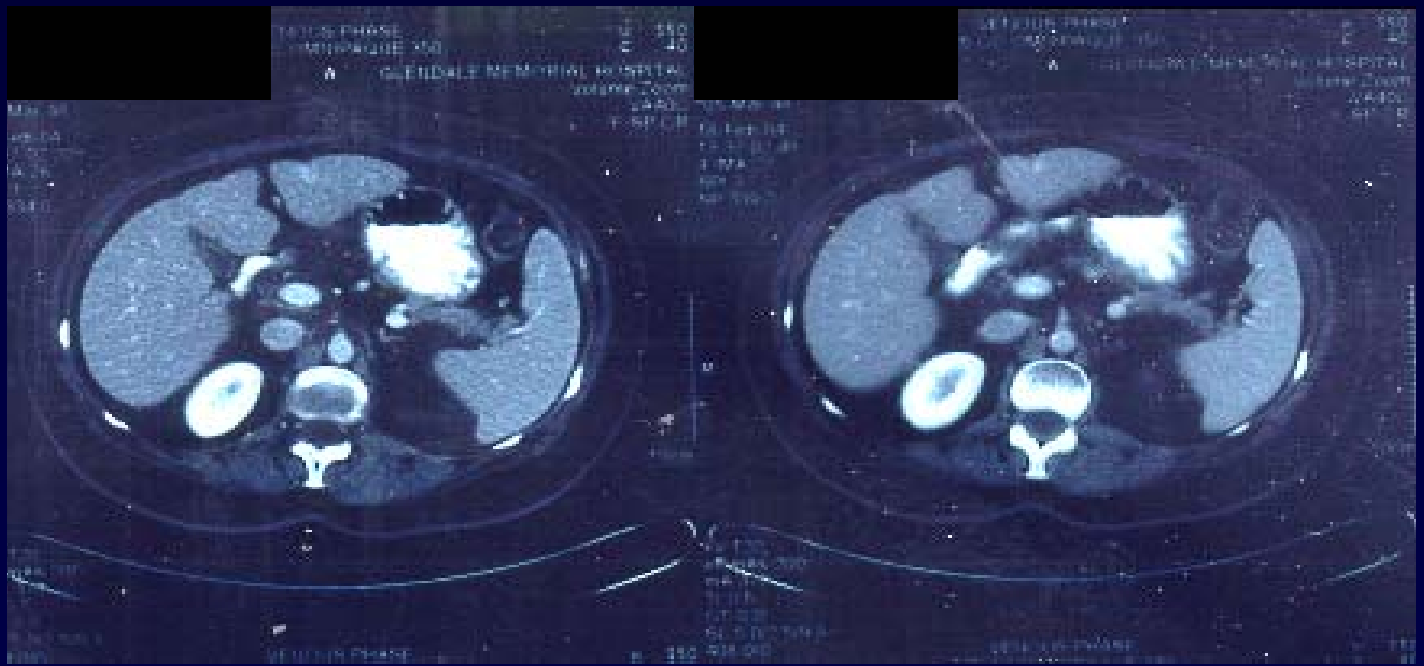


Pharmacokinetics:

Mean $t_{1/2}$ bevacizumab = 19.3d
Mean $t_{1/2}$ trastuzumab = 22.2d

Trastuzumab + Bevacizumab, Phase I


MISSAQUE 350
GLENDALE MEMORIAL HOSPITAL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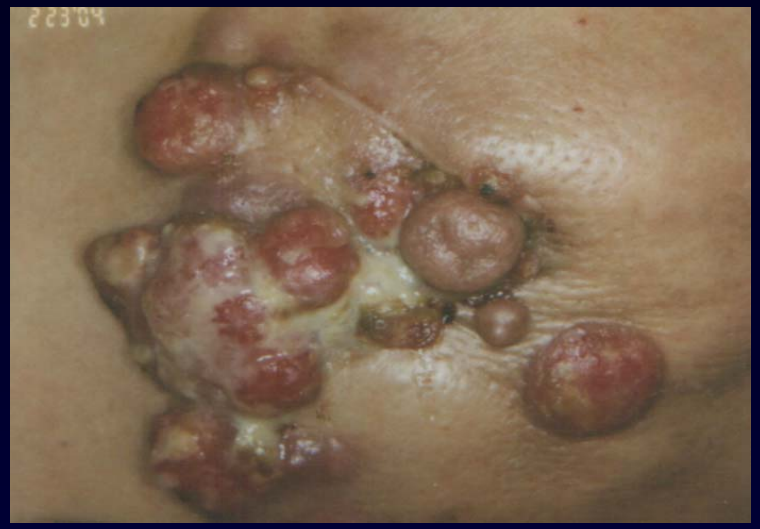
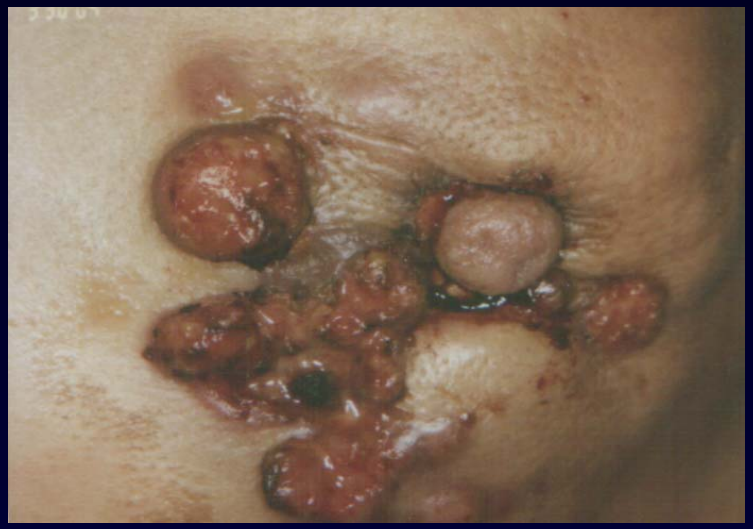
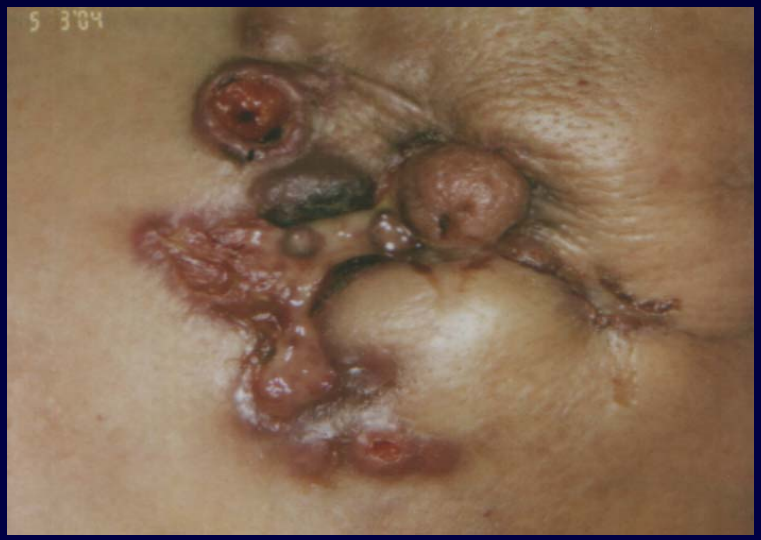


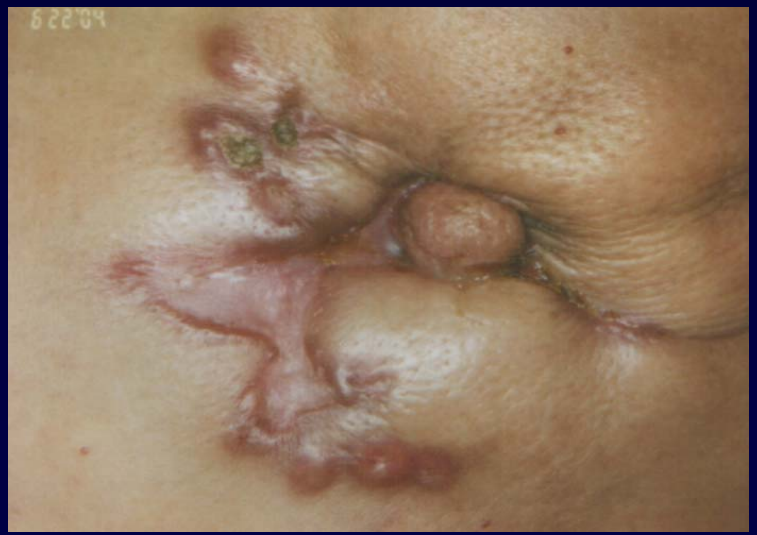
2-23-04



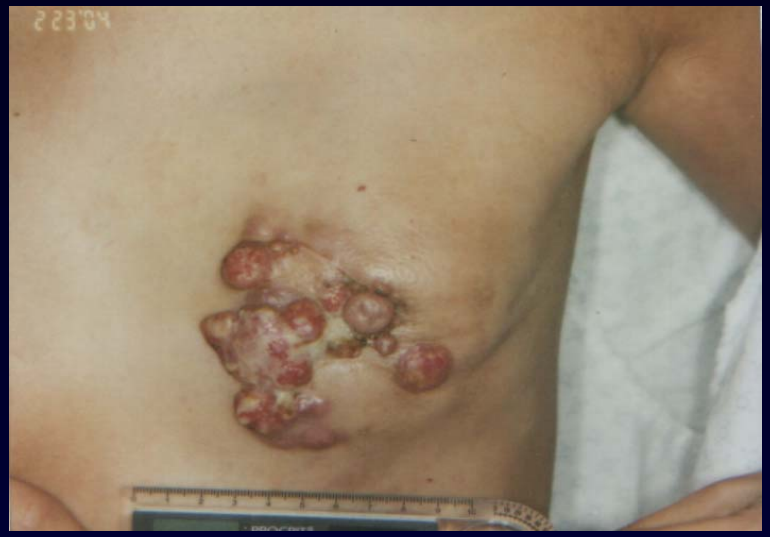
3-30-04



5-3-04



6-22-04



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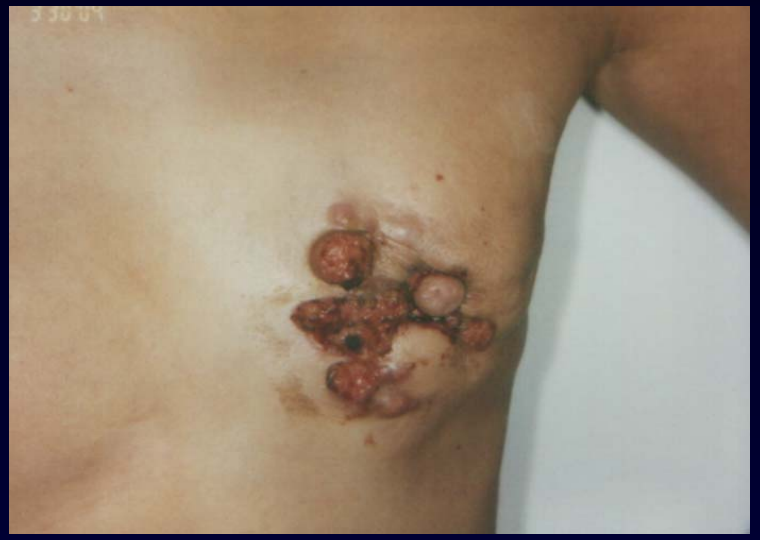

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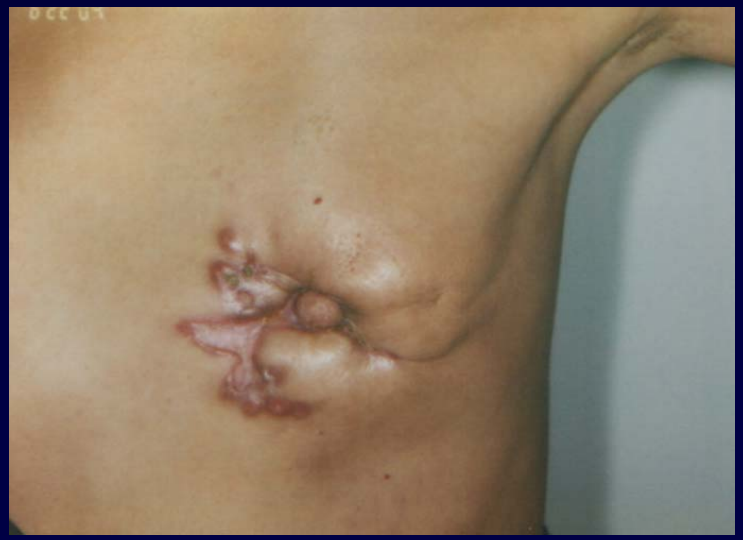

6-22-04

PK/Toxicity/Efficacy Data in 9 pts

- No change in the PK of either antibody when used as combo
- No untoward toxicity induced by combo - 1 pt with mild ^bp treated with diazide
- 2 CR’s
- 3 PR’s
- 2 SD’s > 7 months
- 2 PD’s

Challenges to combined use of targeted therapeutics

- Identifying the appropriate patient population
- Do we simply integrate new targeted therapies with established regimens? Advantages/Problems
- Is broader target specificity better than more narrow targeting?
- What are the most rational targeted combinations to test clinically?
- Can we determine the best likely combinations preclinically before going into the clinic?

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