Brain Metastasis of Breast Cancer: Molecular and Preclinical Advances

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Laboratory of Molecular Pharmacology
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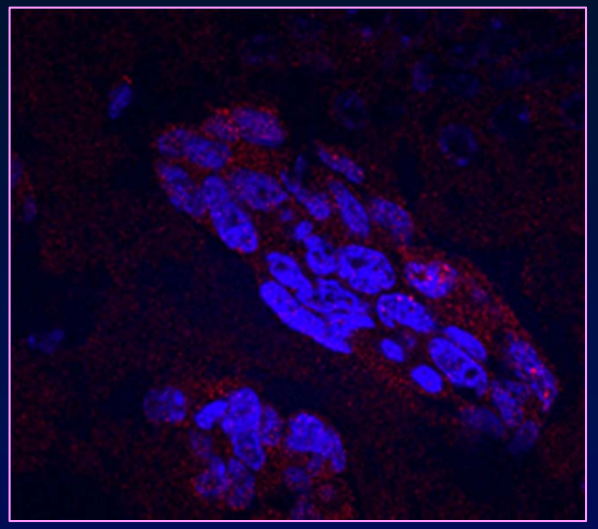
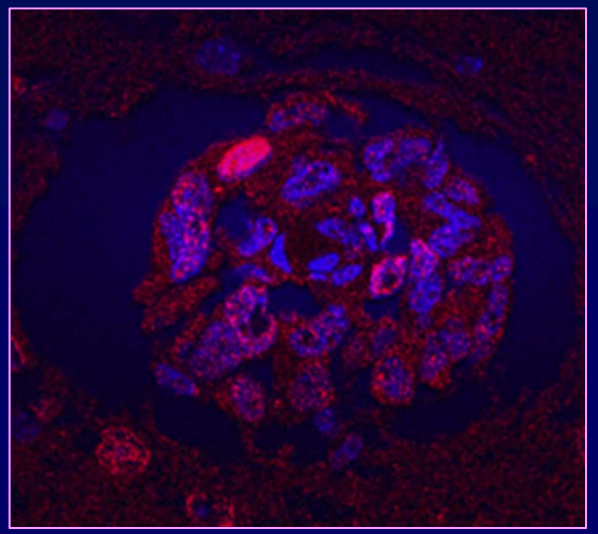




The Problem of Brain Metastases

- **Incidence estimated at 170,000 / year in USA, ten fold higher than incidence of primary malignant brain tumors.**
- **Most commonly arise from metastatic tumors of the lung (50-60%), breast (15-20%), skin (melanoma) (5-10%), and GI tract (4-6%).**
- **Incidence is thought to be increasing. May be a function of an aging population, better treatment of systemic disease, and improved imaging.**
- **In breast cancer, most prevalent in triple negative and Her-2+ subpopulations. Approximately one-third of all metastatic Her-2+ patients now develop brain metastases. Most now develop brain metastases when they have stable disease or are responding to treatment.**
- **Mainstay of treatment is either surgery or stereotactic radiation, +/- whole brain radiotherapy. Limited role for chemotherapeutics.**
- **Significant quality of life issues.**
- **Considered a "sanctuary site" due to blood-brain barrier limited permeability.**

J. Clin. Pathol. 58: 237, 2005
Clin. Cancer Res. 13: 1675, 2007
Am. J. Pathol. 167: 913, 2005; *Clin. Cancer Res.* 13: 1675, 2007; *NEJM* 357:664, 2007; *J. Neuro-Oncology 78:277, 2006; J. Neuro-Oncology 76:59, 2006; Cancer 109: 306, 2007; Cancer 107: 1348, 2006; Lung Cancer* 47: 129, 2004; *Lung Cancer* 46:255, 2004

The Way Things Were:

Primary breast tumor

↓

Metastases in the lung, liver, bone

↓

Brain Metastasis
- After development of other mets
- Near the time of death
- Palliative care only

↓

Death due to systemic disease

What Has Changed:

Better systemic therapy:
- Patients living longer
- More patients responding at metastatic sites or have stable disease

- Brain often the first site of relapse
- Patients need treatment
- Neurocognitive complications

For patients with brain mets, up to 50% of deaths due to CNS disease

Mouse Model of Brain Metastasis of Breast Cancer

MDA-MB-231 “Brain Seeking” (231-BR) tumor cells selected after six rounds of in vivo passage. Cells labeled with EGFP

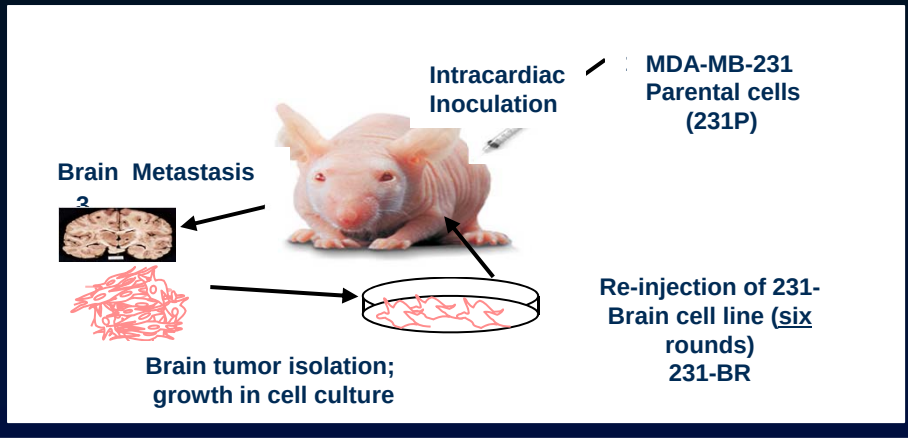
Injection into left cardiac ventricle

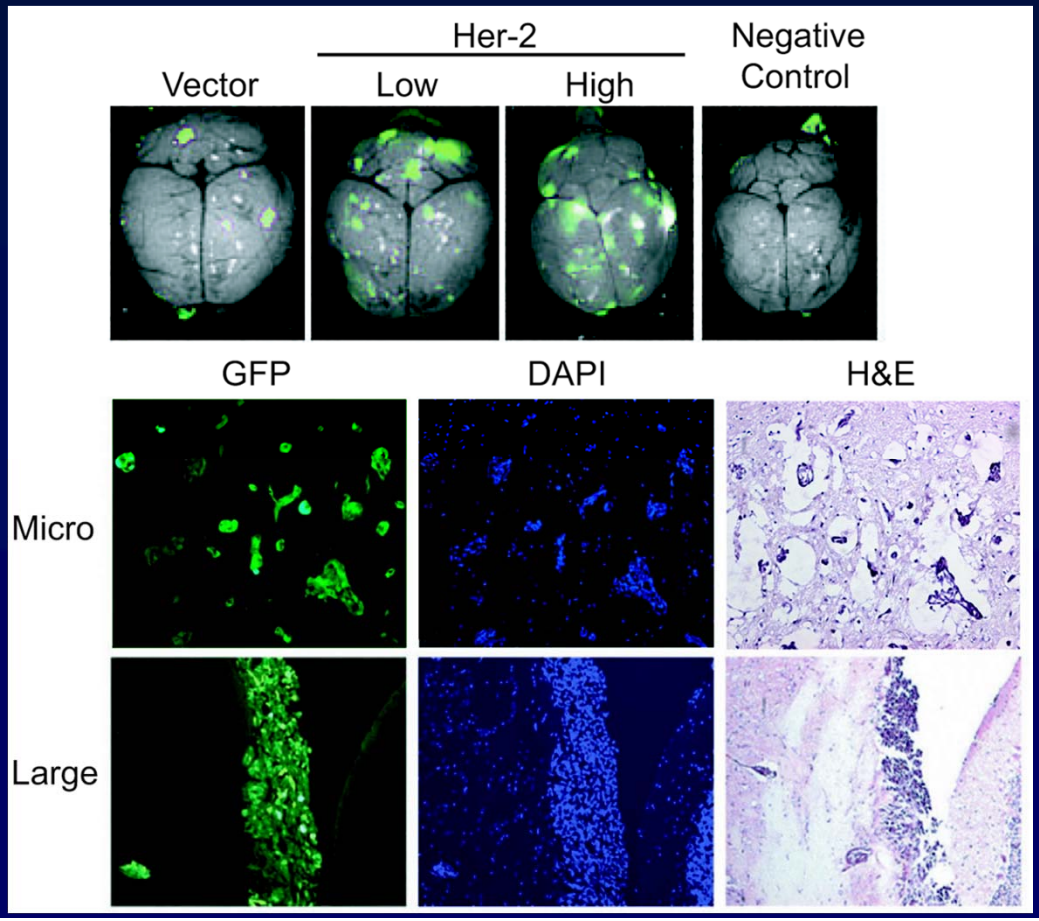
↓ 21-28 days

Imaging
Ten saggittal sections every 300 µM through one hemisphere
- Micromets
- Large Mets

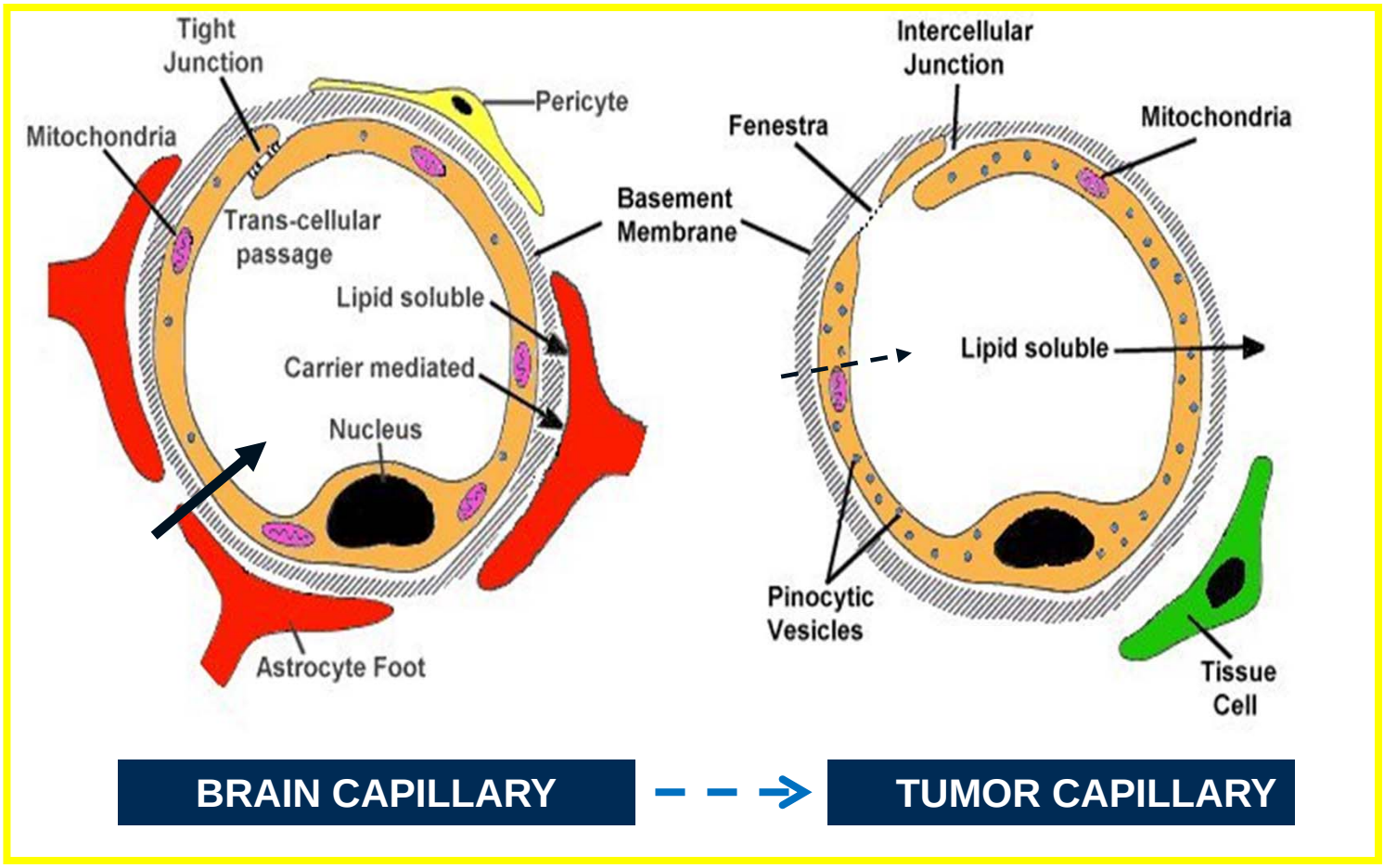
Similar Ki67, apoptotic and neuro-inflammatory response to cohort of craniotomy specimens.

Cancer Res. 67:4190, 2007
Clin. Exp. Metast. 25: 799, 2008.



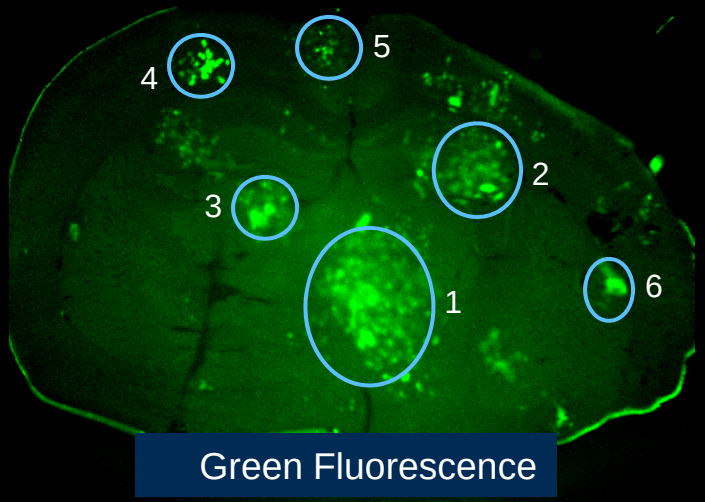


Special Problem of Drug Delivery To Brain Metastases

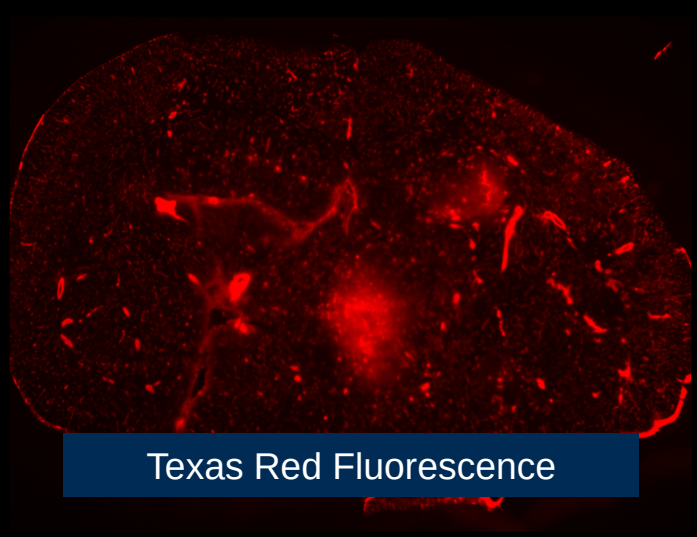


Lesson 1: Blood-Tumor Barrier Passive Permeability is Variably In Experimental Metastases

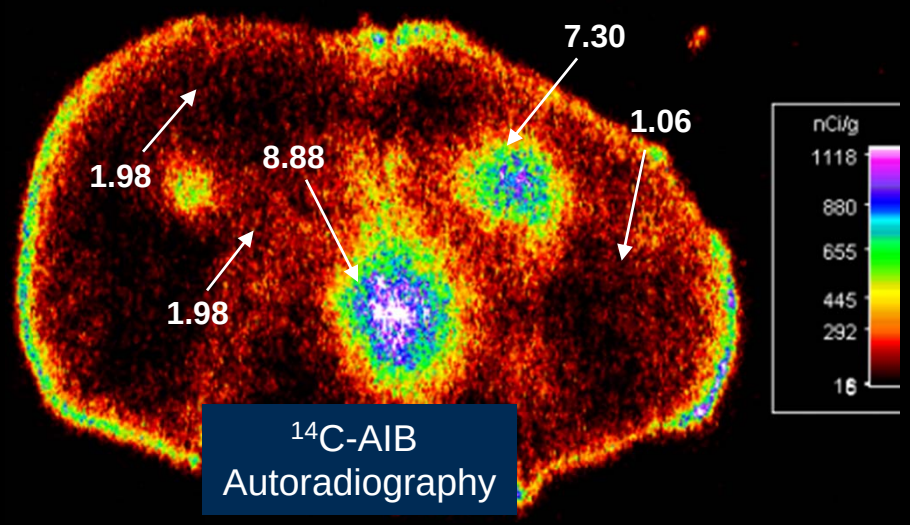
Intracardiac MDA-MB-231BR– 4 wk; 1.5 mg Texas Red 3kD Dextran, 25 µCi ^{14}C-AIB iv – 10 min circulation



Green Fluorescence

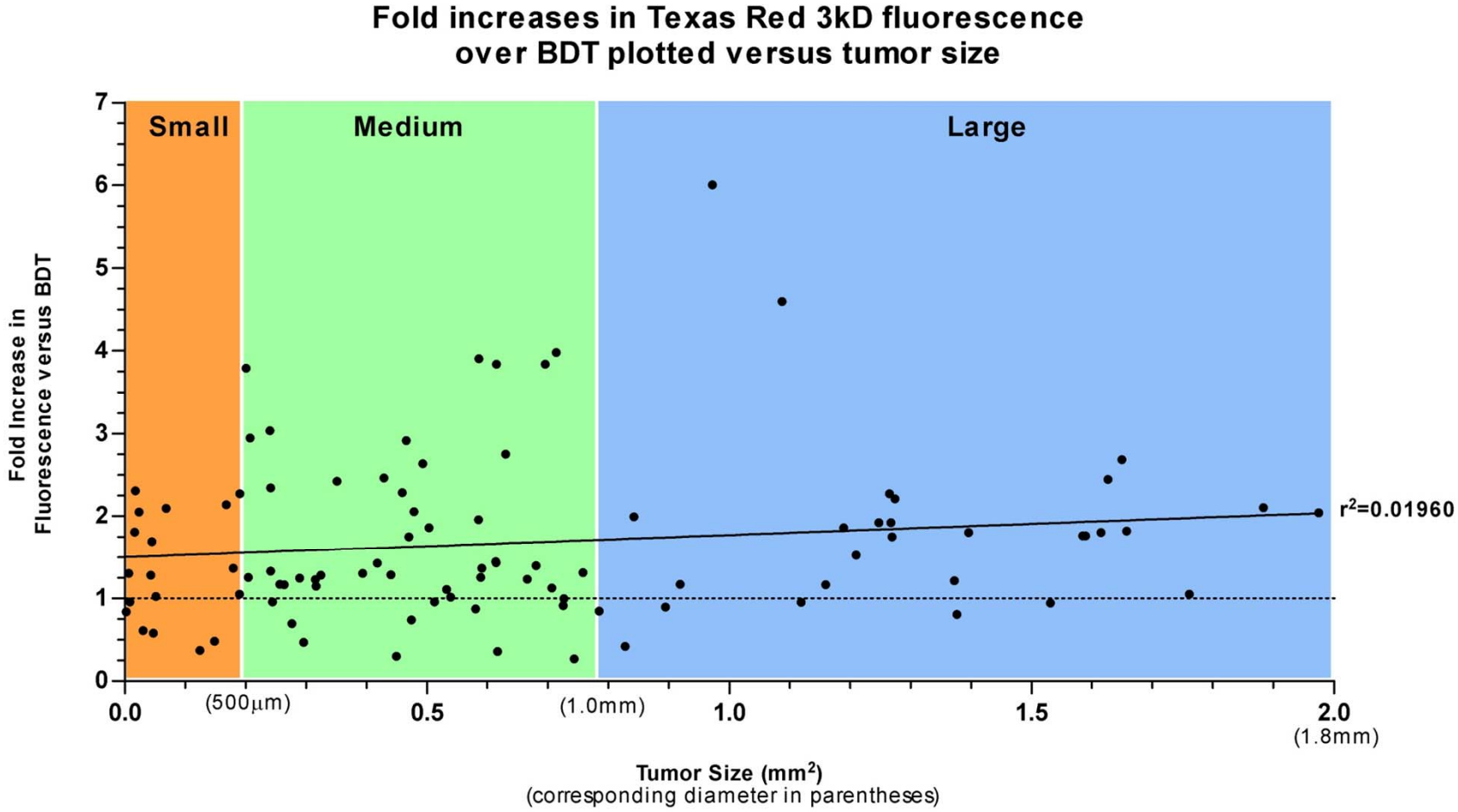


Texas Red Fluorescence



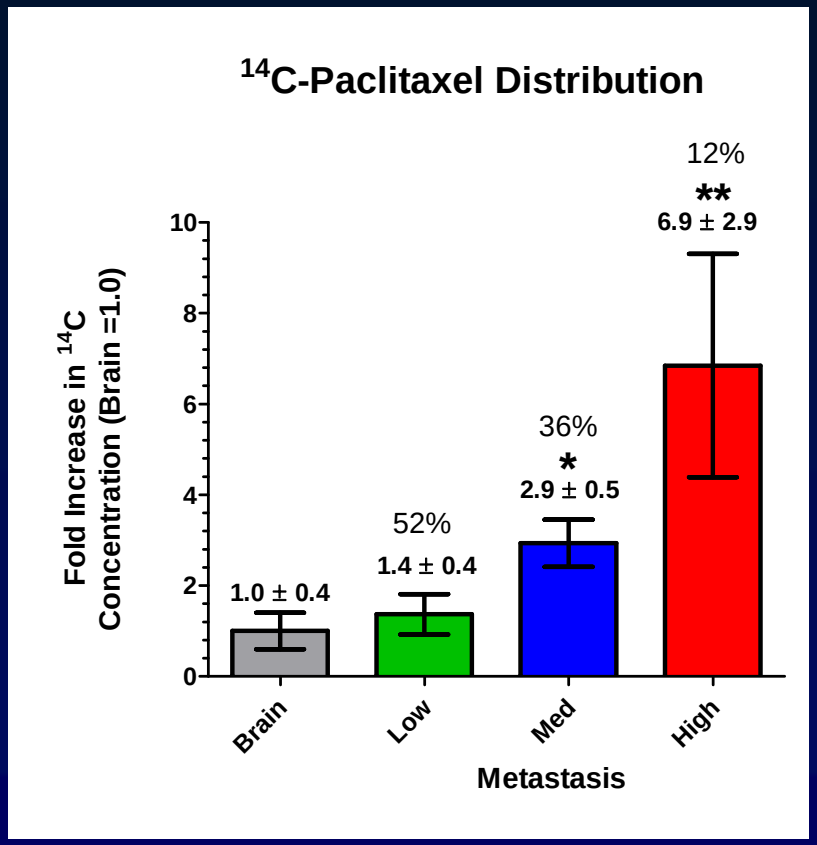
^{14}C-AIB Autoradiography

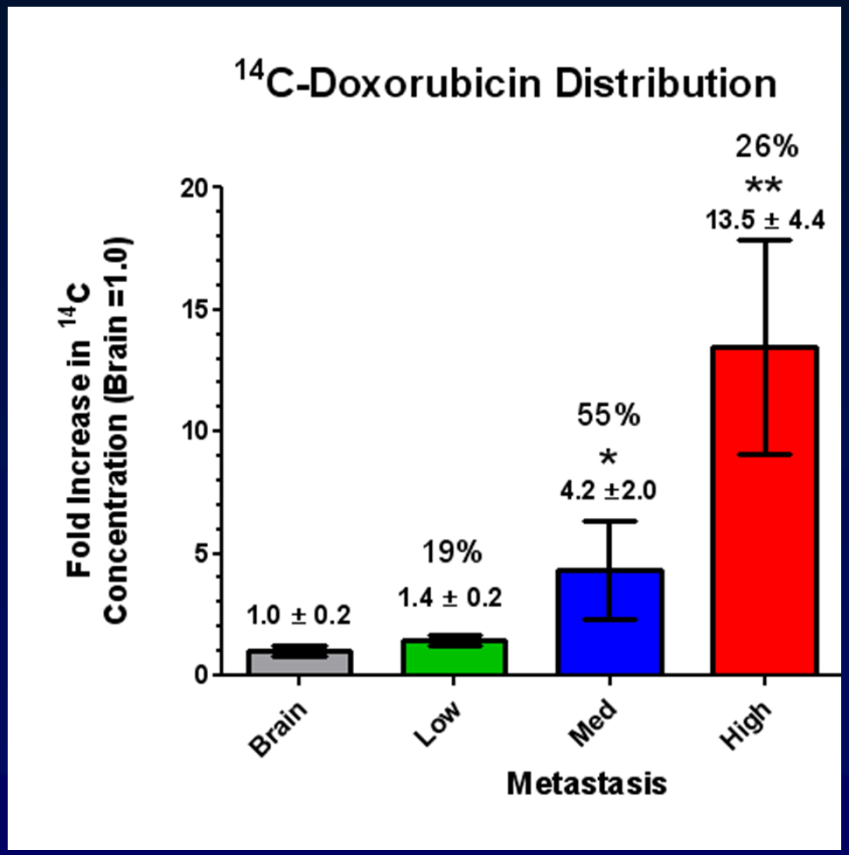
Lesson 2: The BBB is Not “Opened” with Increasing Size of Experimental Metastases



Quentin Smith Ph.D., Paul Lockman Ph.D., Texas Tech

Lesson 3: Heterogeneous and Distinct Patterns of Drug Uptake are Observed in Experimental Metastases





Presurgical Study Open at Cleveland Clinic to Confirm in Human Tumors

Quentin Smith, Ph.D., Paul Lockman, Ph.D.
Texas Tech University

Her-2 Pathway

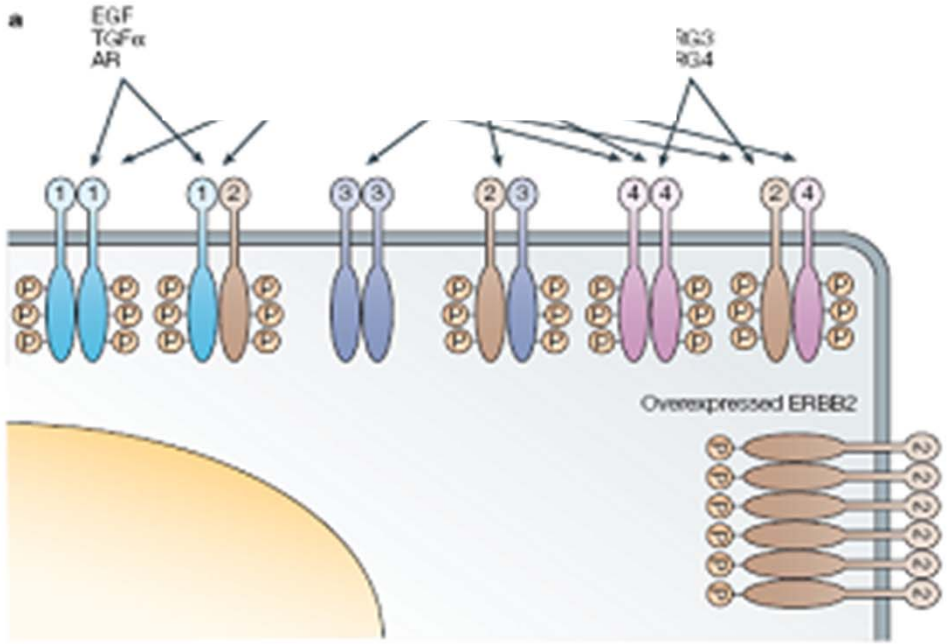
Amplified in approximately 20-25% of breast cancers.

Signals primarily through heterodimerization.

Trastuzumab (Herceptin) is a humanized recombinant monoclonal antibody to Her-2.

Trastuzumab has efficacy in combination with chemotherapy in the metastatic and adjuvant settings.

A high proportion of metastatic Her-2+ breast cancer patients are developing brain metastases



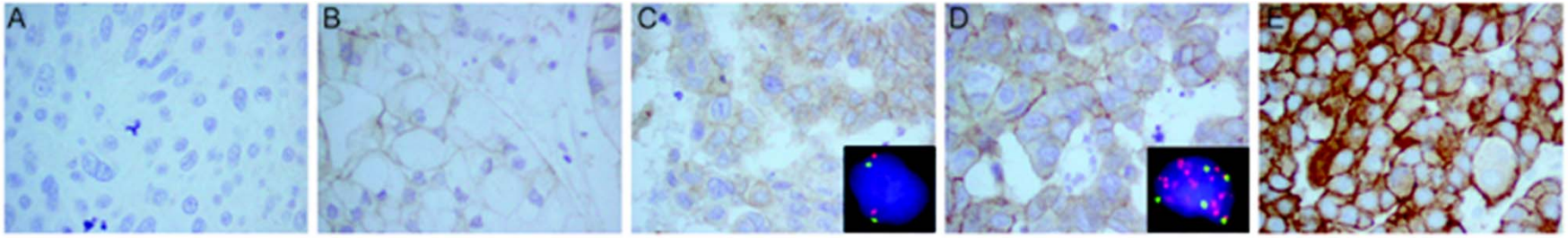
Nature Rev. Cancer 5:341, 2005

Her-2 Status and the Development of Brain Metastases

Of 93 metastatic patients receiving Herceptin, brain metastases occurred in 25% over a median followup time of 10.8 months. 78% of patients with brain metastases had stable disease at other sites. The CNS was the first site of symptomatic progression in 82% of patients, and the only site of disease progression at that time in 69% of patients. 50% of patients died from their CNS disease.

Br. J. Cancer 91:639, 2004

Her-2 is Frequently Overexpressed in Resected Brain Metastases

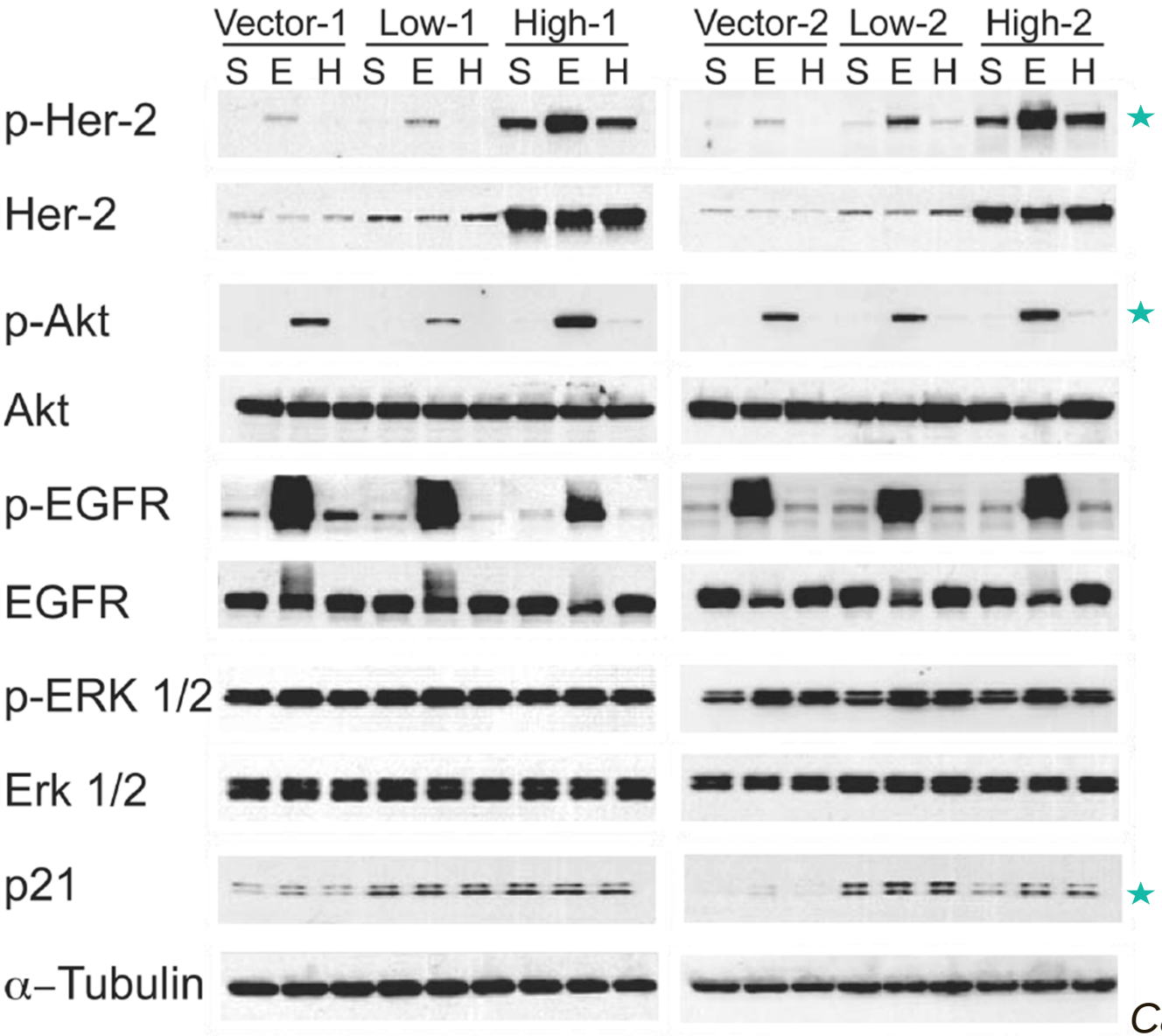


Intensity	0	1+	2+ (FISH -)	2+ (FISH +)	3
Number	21	36	22	5	40
Percent	16.9%	29%	15%	36.2%	

Kenneth Aldape
Department of Neurosurgery, MDACC

Cancer Res. 67:4190, 2007

Her-2 Transfectants of the 231-BR Cell Line



Increased
- pHer-2
- pAkt
- p21

Increased Colonization
Equivalent Proliferation,
Endothelial invasion.

Cancer Res. 67:4190, 2007

Her-2 Overexpression Promotes the Metastatic Colonization of the Brain by Breast Cancer

Clone:	N:	Mean (95% Confidence Interval) Metastases: Micromets:	Large:	P:
Vector 1	8	250.5 (207 - 293)	**5.1 (3.7 - 6.6)**	
Vector 2	5	80.5 (92 – 267)	**2.9 (2.0 – 3.8)**	
Low Her-2 1	7	145.5 (102 – 286)	**11.3 (8.3 – 14.4)**	
Low Her-2 2	11	194.5 (159– 230)	**16.6 (15.1 – 18.1)**	**0.0001**
High Her-2 1	9	182.9 (141– 226)	**10.9 (8.9 – 12.9)**	
High Her-2 2	5	254.0 (175 – 336)	**14.0 (11.6 – 16.4)**	**0.0001**

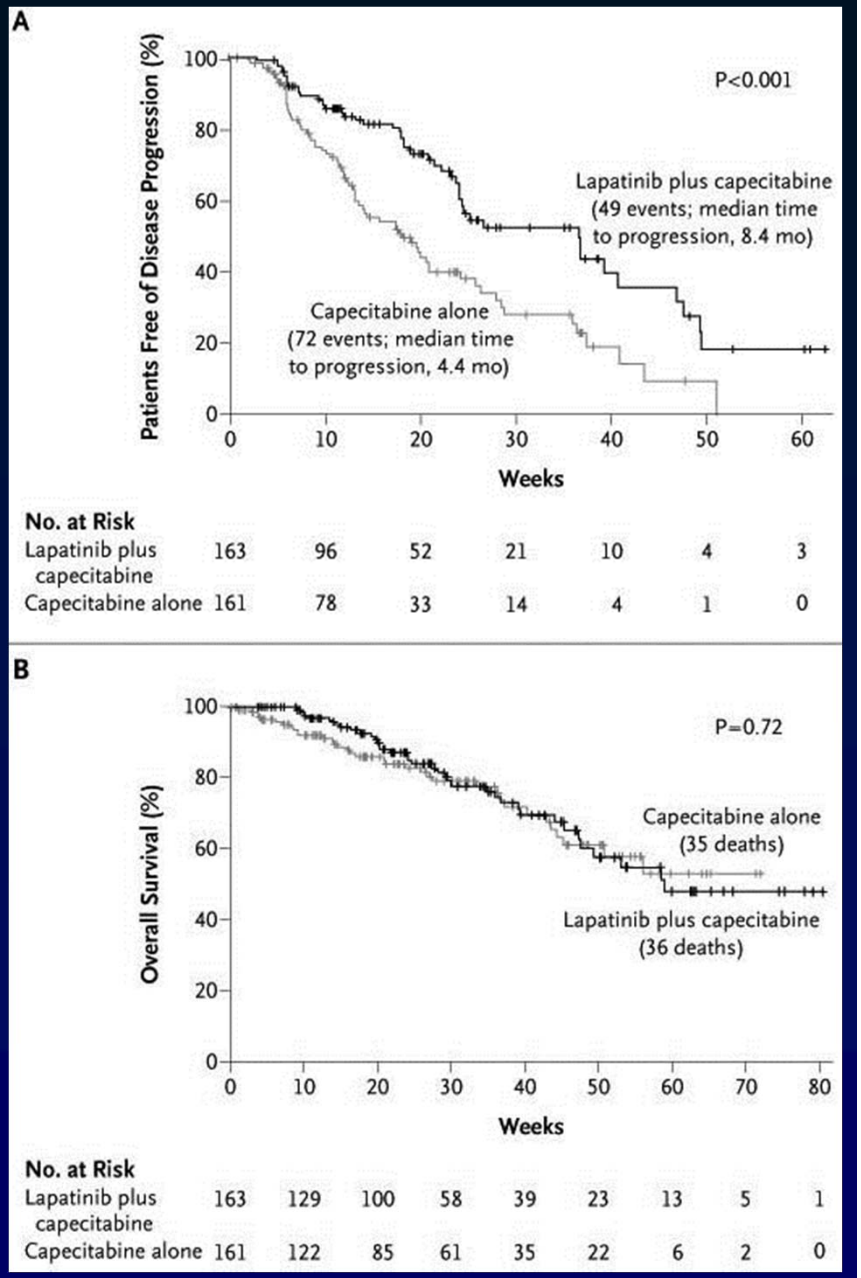
Her-2 overexpression alters the natural history of breast cancer to promote brain metastatic colonization.

Cancer Res. 67:4190, 2007

Lapatinib

- Orally available tyrosine kinase inhibitor of EGFR, Her-2.
- Most clinical activity directed to Her-2 in breast cancer to date
- FDA approved in combination with capecitabine for patients with Her-2+ tumors who have received prior trastuzumab.

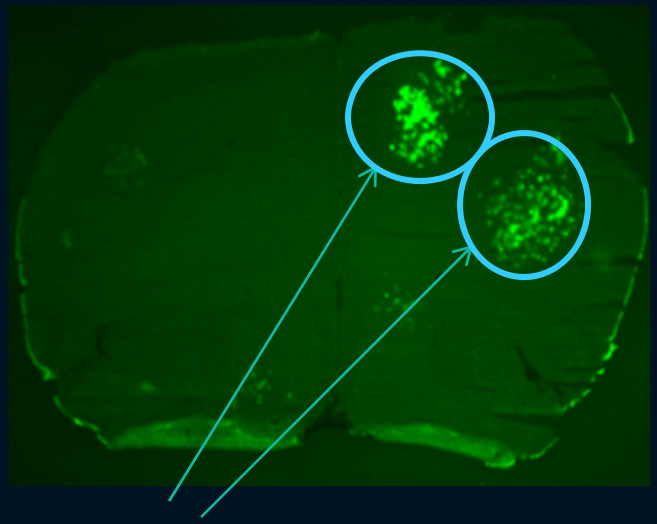
Hypothesis: Lapatinib will prevent the metastatic colonization of Her-2+ breast cancer in the brain.



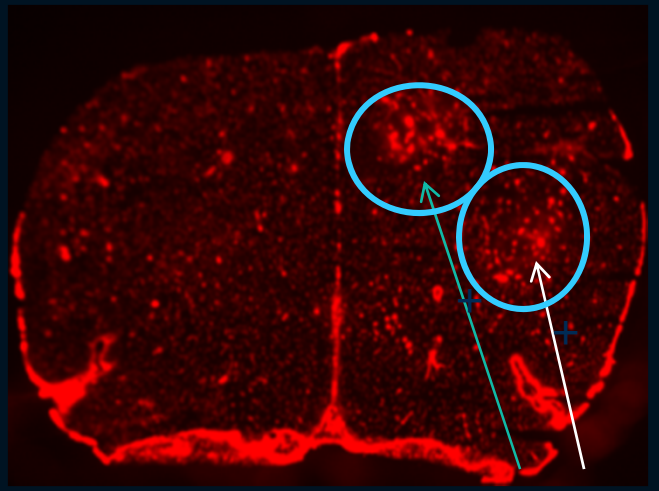
JCO 23:5305, 2005
NEJM 355:2733, 2006

^{14}C-Lapatinib Distribution in Brain Metastases

Green Fluorescence – GFP Tumor

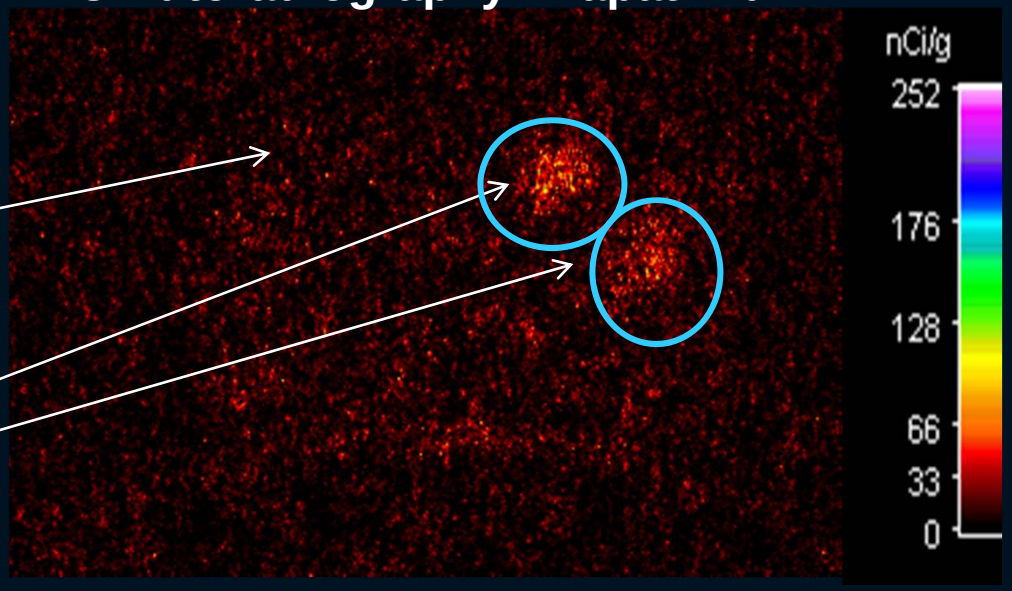


Red Fluorescence – Texas Red 3kD Dextran



Texas Red Leakage

^{14}C Autoradiography - Lapatinib



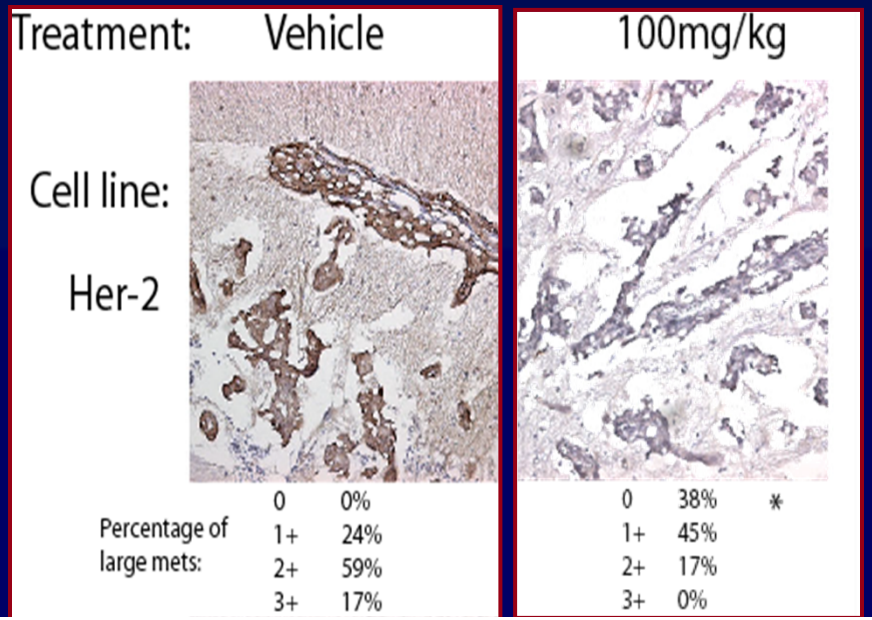
1.5 nCi/g
Normal Brain

23-26 nCi/g
Tumor

	2 hr	12 hr
Blood (µM)	5.5	1.0
Brain (µmol/kg)	0.15	0.05
Brain metastases (µmol/kg)	2.0	1.0
Systemic Metastases (µmol/kg)	----	3.4

Lapatinib Inhibits Brain Metastatic Colonization

Cell Line:	Treatment:	N	Mean (95% Confidence Interval) Metastases: Micrometastases:	Large Metastases:
Her-2+	Vehicle	22	138 (102-175)	**6.83 (5.9 - 7.8)**
	30 mg/kg	25	109 (72-146)	**3.21 (2.3 – 4.1)*** **
	100 mg/kg	26	127 (90-164)	**3.44 (2.6 – 4.3)*** **



*** P < 0.0001

JNCI 100: 1092, 2008

The Clinical Data are Consistent with a Preventive Effect

Lapatinib for Treatment of Brain Mets- Phase II Trial Results:

1 PR/39 patients
Some stable disease noted

***J. Clin. Oncol.* 26: 1993, 2008**

Lapatinib Prevention of Brain Mets- Phase III randomized trial of Lapatinib + Capecitabine vs. Capecitabine:

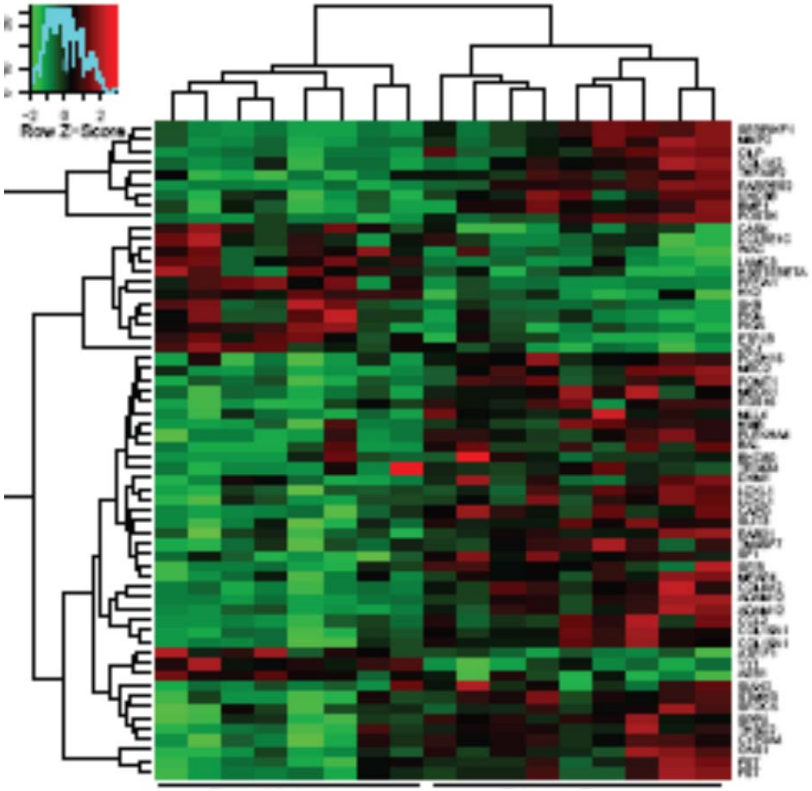
Significant reduction in CNS mets as first site of relapse (P=0.045).

***Br. Ca. Res. Trt.* 112: 533, 2008**

Further testing in the adjuvant Trans-ALTTO and TEACH trials

Gene Expression Analysis of Primary Breast Tumors And Resected Brain Metastases of Breast Cancer:

80% of Differentially Expressed Genes are Down-regulated in Brain Metastases



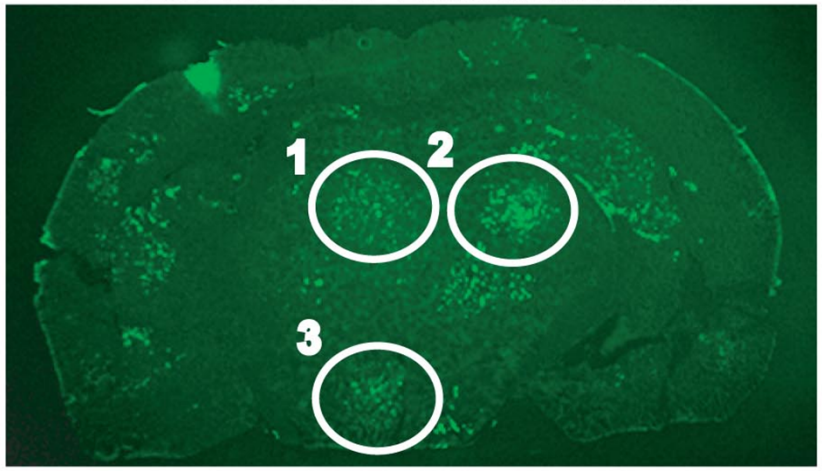
QRT-PCR Validation of Differentially Expressed Genes.

Gene:	Trend on Microarray[1]:	Mean (95% Confidence Interval) [2]		p value[3]
		Brain Metastasis	Primary Tumor	
BHC80	Down in BM	22.5 (11.7-33.3)	63 (25.1-100.9)	0.034
BMP1	Down in BM	2.6 (1.67-3.52)	7.8 (3.6-12.0)	0.014
HK2	Up in BM	1.0 (1.47-0.57)	0.59 (0.74-0.45)	0.097
LOXL1	Down in BM	3.83 (2.55-5.11)	8.97 (3.2-14.74)	0.062
PEDF	Down in BM	5.35 (0.71-11.41)	7.60 (4.41-14.760)	0.068
SIAH	Down in BM	1.01 (0.83-1.18)	6.63 (4.76-8.5)	0.056
SLIT3	Down in BM	1.12 (0.55-1.69)	2.44 (0.92-3.96)	0.087
STHMN 3	Down in BM	0.56 (0.14-0.98)	1.92 (0.56-3.28)	0.048
TSPD2	Down in BM	0.13 (0.02-0.24)	2.71 (0.91-4.51)	0.005

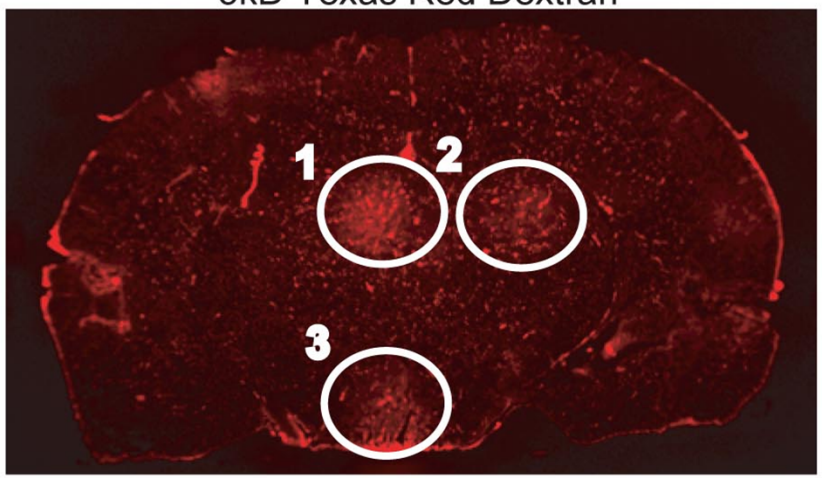
Mol. Cancer Res. 7: 1438, 2009.

Hypothesis: A HDAC Inhibitor Will Alter Gene Expression Pattern and Metastatic Capacity
Vorinostat (SAHA) Shows Overall Brain Permeability

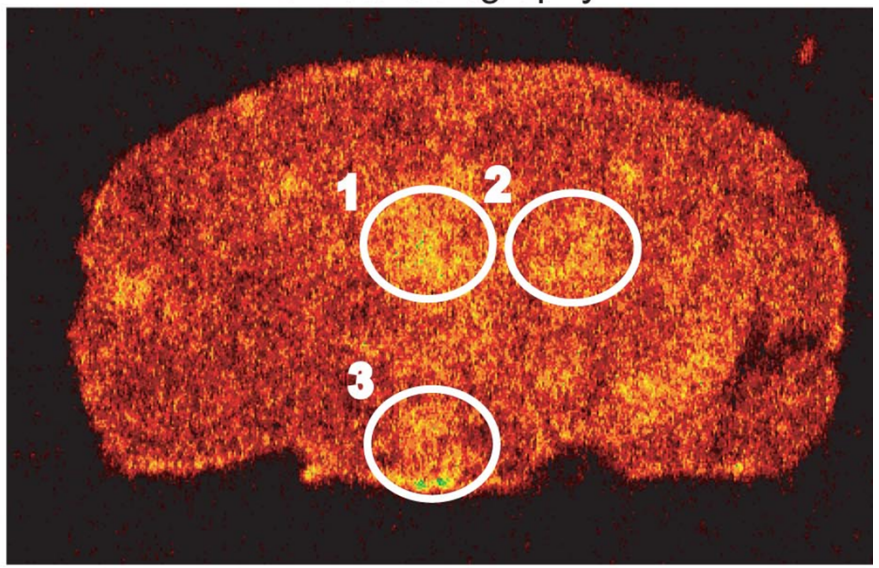
231-BR-EGFP Brain Metastases



3kD Texas Red Dextran



^{14}C Autoradiography



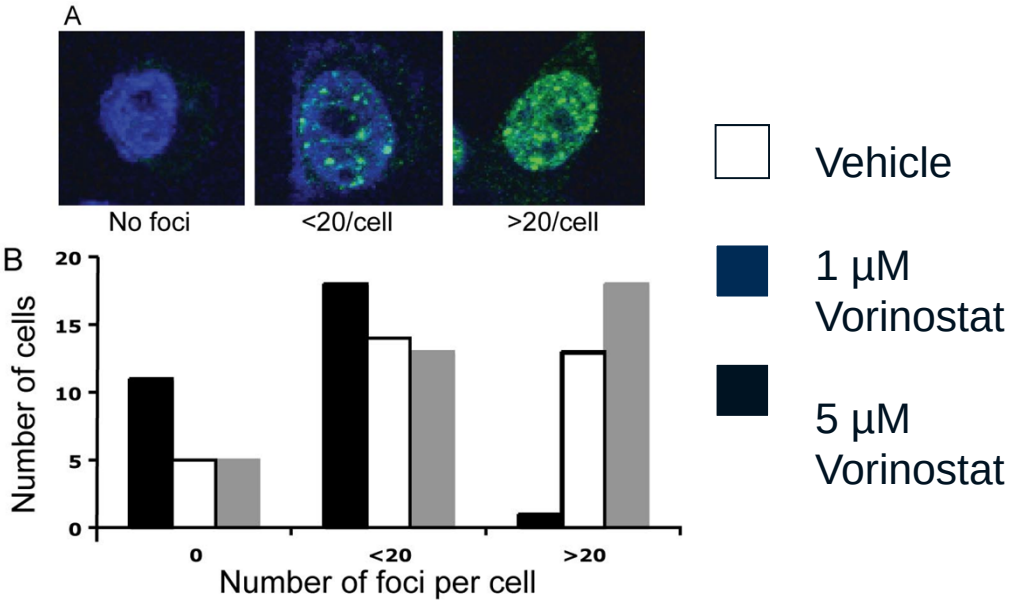
Clin. Cancer Res. 15: 6148, 2009.

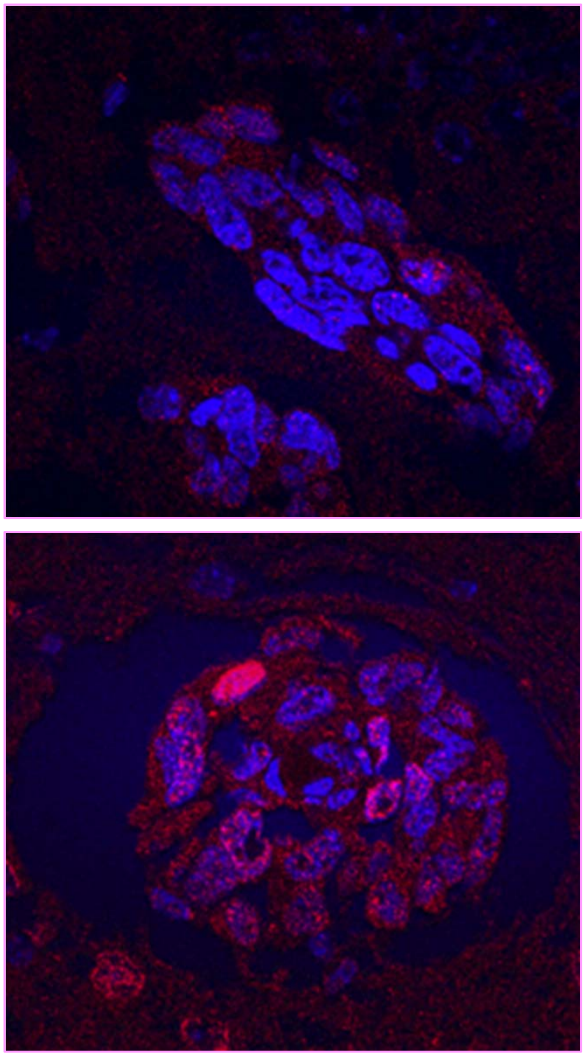
Vorinostat (SAHA) Prevents the Development of Experimental Brain Metastases When Administered Early

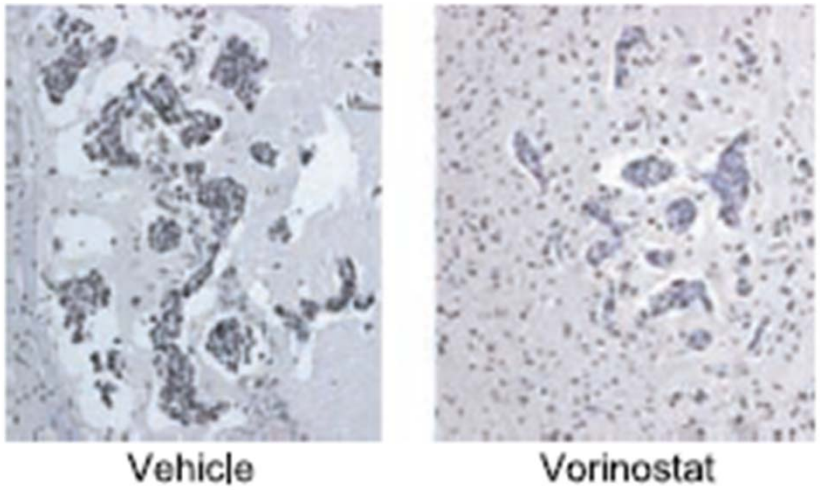
Treatment	N	Micrometastases:			Clinical Metastases:		
		Mean	95% CI	P	Mean	95% CI	P
Vehicle	20	170	146-193		7.65	6.20-9.10	
SAHA D3 post-injection	18	122	98-146	0.017	2.89	1.94-3.84	< 0.0001
SAHA D7 post-injection	19	151	127-176	NS[3]	4.94	3.90-5.98	0.008
SAHA D14 post-injection	18	177	153-201	NS	5.96	4.69-7.22	NS

Clin. Cancer Res. 15: 6148, 2009.

Vorinostat Induced Double Strand DNA Breaks In Vitro and In Vivo



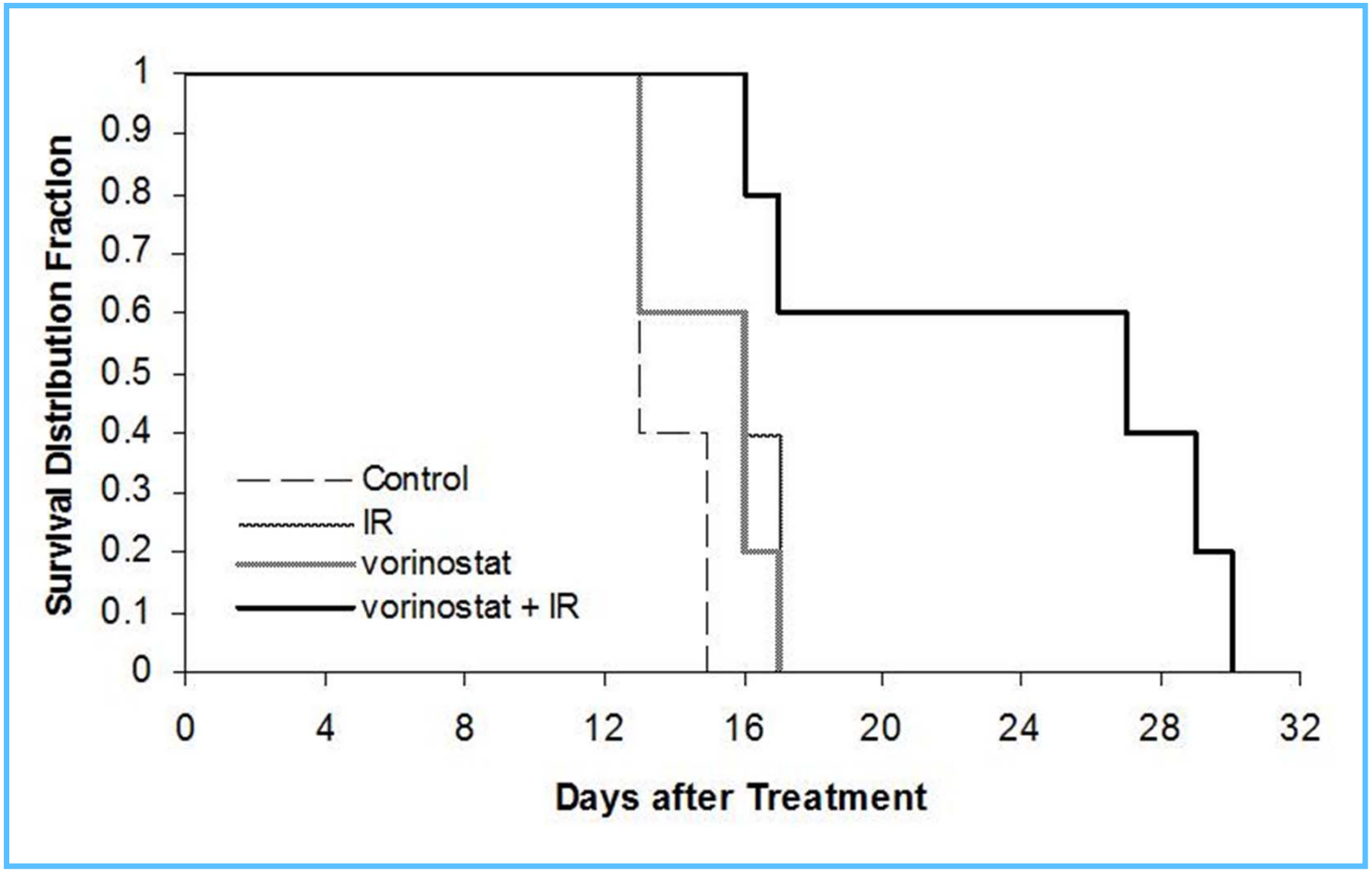




Rad 54 IHC

Clin. Cancer Res. 15: 6148, 2009.

Vorinostat Synergized with Radiation to Increase Survival In Vivo Intracranial Implantation



Log rank test, p=0.038

Phase II Trial of Vorinostat + Stereotactic Radiosurgery or WBRT
Thomas Jefferson University
Adam Dickler,M.D., PI

Kevin Camphausen
Mol. Cancer Ther. 8: 1589-1595, 2009.

Brain Metastases in Breast Cancer Workshop

March 1-2, 2009

Ritz Carlton
Arlington, VA

Sponsored by

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www.rtog.org

DAY 1
5:00 – 9:00 PM

PART I: INTRODUCTIONS AND OVERVIEW

Welcome	Nancy Lin, MD Minesh Mehta, MD
Dinner	
General Overview	Eric Winer

PART II: PRESENTATIONS

PRE-CLINICAL DATA

Preclinical Data Overview	Pat Steeg, PhD
Preclinical Data on Brain Permeable Compounds	Quentin Smith, PhD
Signatures for Brain Relapse	Sunil Badve, MBBS, MD

CLINICAL DATA AND LESSONS LEARNED

ENRICH Trial	John Suh, MD
Motexafin Trial	Minesh Mehta, MD
Lapatinib Trials	Nancy Lin, MD
Epothilone Trials	David Peereboom, MD
Angiogenesis Inhibitors for Brain Tumors/Mets	April Eichler, MD

DAY 2
8:00 AM – 2:00 PM

PART III: PRESENTATIONS

OVERVIEW OF TRIAL GROUPS/CONSORTIA

Rapid Early Stage Trials: Unique Designs	Morris Groves , MD, JD
TBCRC	Eric Winer, MD
P2C	Jamie Zwiebel, MD
RTOG	Walter Curran, Jr, MD
Opportunities & Resources for Molecular Imaging in Clinical Trials	Lalitha Shankar, MD, PhD
Novel Imaging Methods	A. Gregory Sorenson, MD

SURGERY AND RADIOSURGERY

Surgical Considerations	Fred Lang, MD
Radiosurgery (incl. multiple lesions) & Necrosis	Ajay Bhatnagar, MD
Role of PCI	Elizabeth Gore, MD

SURROGATE ENDPOINTS

Cognitive Evaluation; Neuroprotective Strategies	Minesh Mehta, MD
Neuro-psych Testing in Multicenter Studies	Jeffrey Scott Wefel, PhD

PART IV: DISCUSSION

Working Lunch/Discussion

Organizers:

JoAnn Zujewski, M.D.
CTEP, NCI
Eric Winer, M.D.,
Harvard
Minesh Mehta, M.D.,
RTOG
Patricia Steeg, Ph.D.,
CCR, NCI

Issues:

- Multi-disciplinary collaboration
- Coordination of preclinical and Clinical trials
- Clinical trial design

Hypothesis: There is a role for drugs in brain metastasis therapy.

Hypothesis: Most molecular therapeutics will prevent, not cure brain metastases.

Prevention Trials:

- Brain permeable drugs with efficacy against micrometastatic disease (prevents metastatic colonization)
  Vorinostat, Lapatinib, Plk1 inhibitor

- "Window of opportunity" trial design: PFS for new metastasis in patients with at least one brain metastasis, who have not received WBRT.

- Reasonable side effect profile

- Can these drugs eliminate need for WBRT?

Therapeutic Trials:

- Brain permeable drugs that synergize with radiation
  Vorinostat

Or, for patients not able to continue on with radiation:

- Brain permeable drugs with cytotoxic activity in brain
  Plk1 inhibitor

Other Agents Under Consideration:
CTEP/CCR Collaboration

- TAK-275, Hg inhibitor, Parp inhibitors, γ-secretase inhibitors, brain permeable microtubule stabilizers
- Preclinical efficacy and PK, and Clinical data side-by-side
- Include “backbone” chemotherapy for systemic disease

BrainMetsBC.org

Understanding brain metastases, available treatments, and emerging research.
A Website for Patients and Families . . .

Musa Mayer
Helen Schiff

Acknowledgements

Women's Cancers Section, LMP, CCR, NCI *:

Diane Palmieri, Brunilde Gril, Dan Fitzgerald, Yong Qian

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Quentin Smith*, Paul Lockman*, Texas Tech University
Kenneth Aldape*, MD Anderson Cancer Center
Paul Meltzer, Sean Davis, Robert Walker, NCI
Maria J. Merino, Surgical Pathology Section, LP, NCI
Seth Steinberg, NCI, Statistics
Kevin Camphausen, Radiation Oncology Branch, NCI

Musa Mayer, Helen Schiff, Lilla Romeo, Patient advocates

GlaxoSmithKline
Merck

*** DOD Breast Cancer Research Program Center of Excellence**