Preclinical Data Overview

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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.**
- **Prognosis poor. 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

Vision

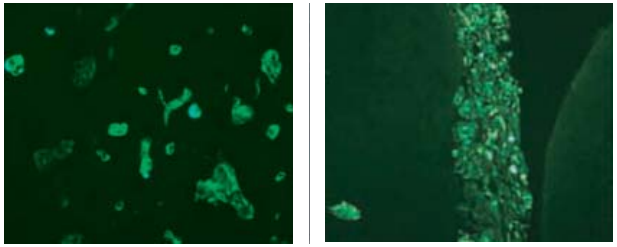
Therapeutic Trials:

- Drugs that synergize with radiation
- Or drugs that will hold micromets in check after gamma knife radiation or surgery

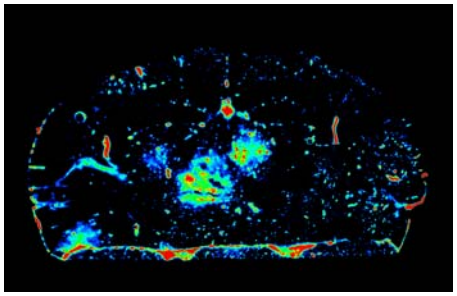
Prevention Trials:

- Must identify patients at high risk
  (Metastatic setting? Gene signature?)
- Brain permeable drugs with some breast cancer efficacy
- Tolerable side effect profile

Validation of a Quantitative Brain Metastasis Model System



Histology



Drug Uptake

- **Derivation of 231-BR Cells: MDA-MB-231 human breast cancer cell line. Brain metastases serially passaged (UTSA).**
- **EGFP labeled.**
- **Injected into left cardiac ventricle. Endpoints are imaging, and histological analysis of sections through one hemisphere.**
- **As compared to the parental cells, 231-BR is less proliferative but more motile.**
- **Compared to a panel of 16 resected human brain metastases to determine relevance to human disease.**
- **231-BR cell line model has now been sent out to 20 labs.**

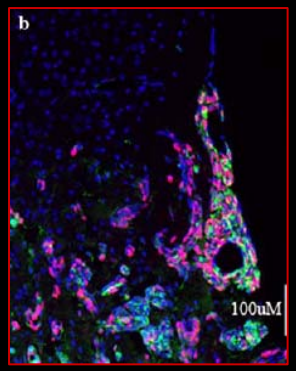
231-BR Model Similar to Human Brain Metastases in Proliferation, Apoptosis and Neuro-inflammatory response.

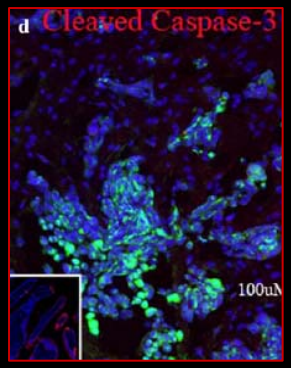
Tumor Cells with DAPI and immunoflourescent markers

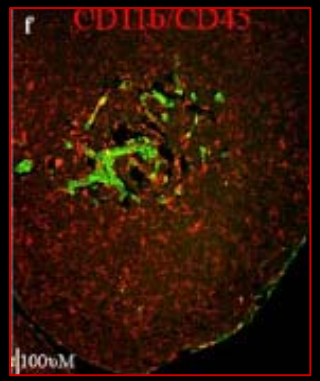
Clin. Exp. Met.., 2008

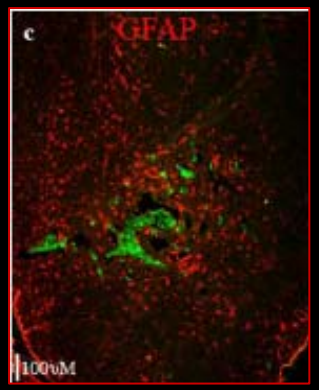
MOUSE

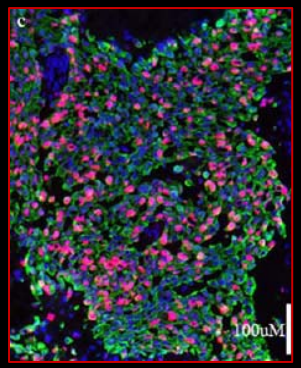
HUMAN

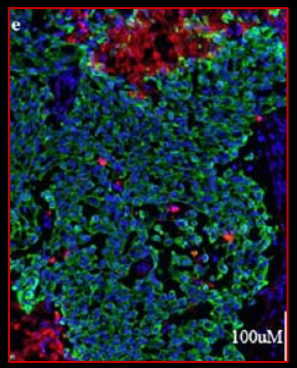


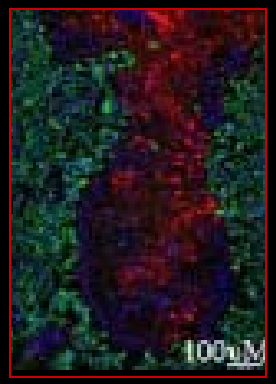


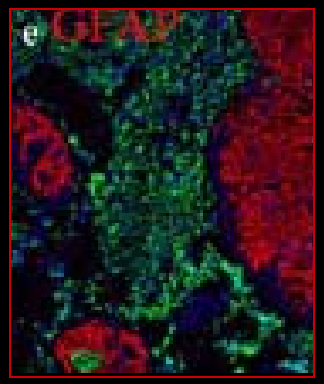






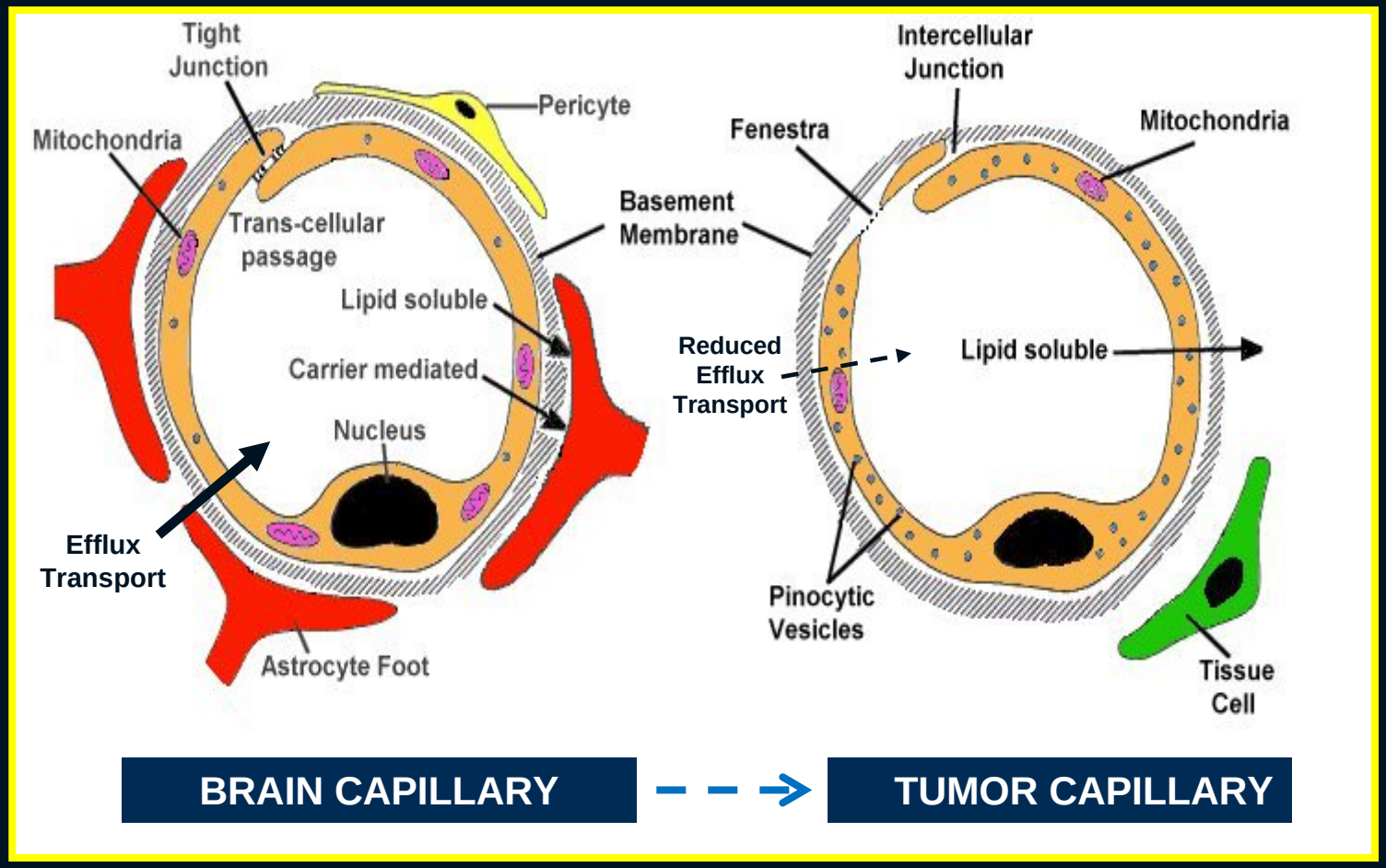






Ki 67 **Cleaved Cas 3** **Microglia** **Astrocytes**

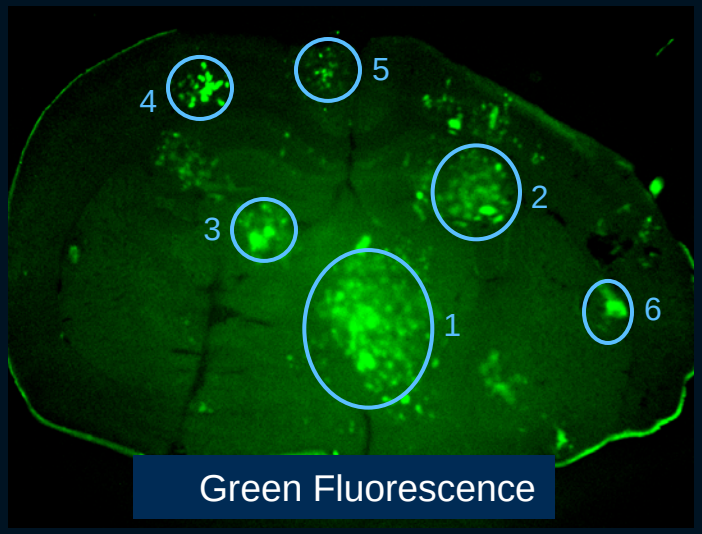
Special Problem of Drug Delivery To Brain Metastases

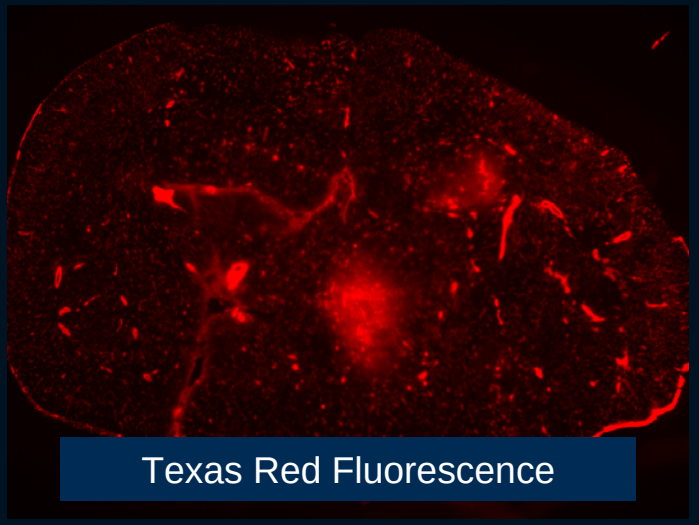


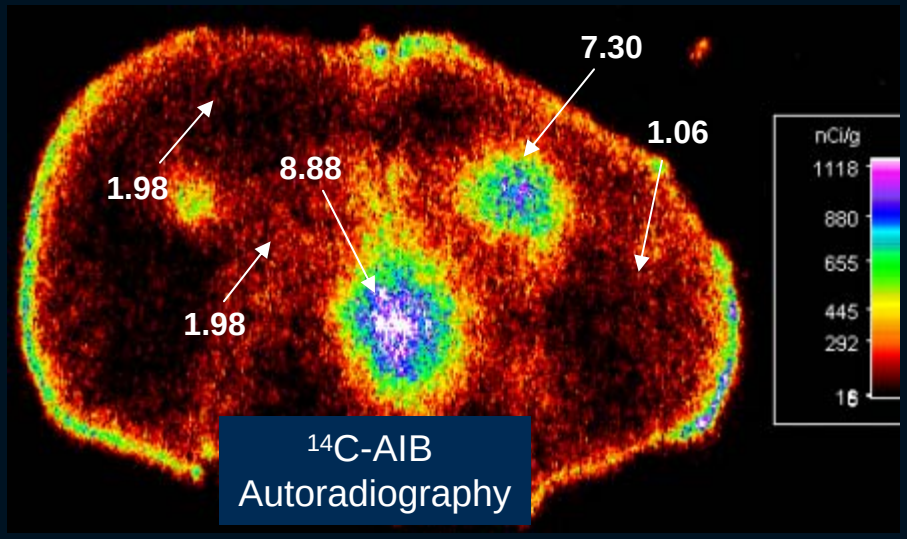
Blood-Brain and Blood-Tumor Barriers

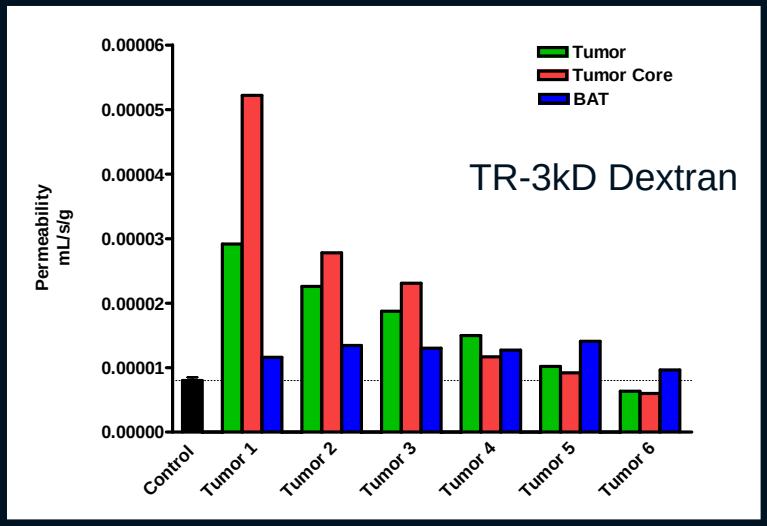
Blood-Tumor Barrier Passive Permeability is Variably Increased in Some Brain Metastases

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



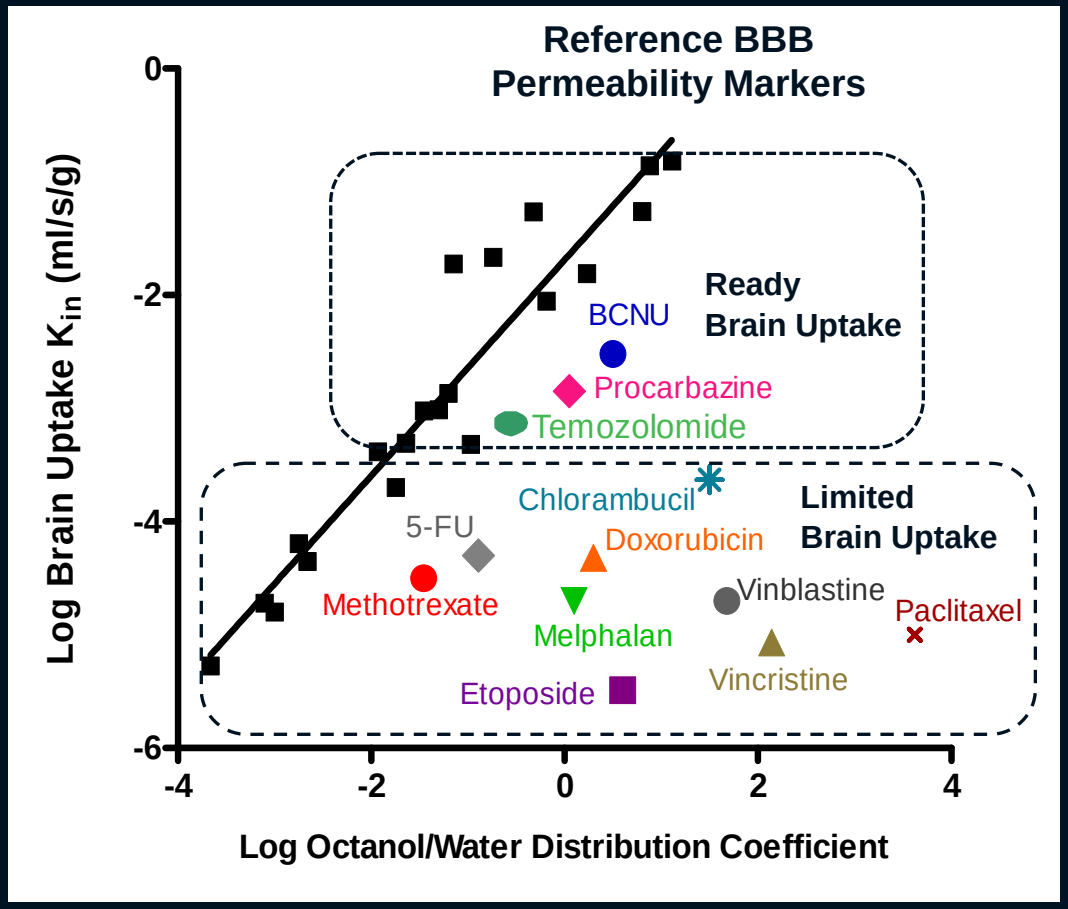






Many Anticancer Drugs Do Not Cross the Blood-Brain Barrier Well

In Situ Brain Perfusion Uptake Measurements



K_{in} = Blood-to-Brain Transfer Constant

Critical Limiting Factors:

1. **Large M W (>500)**
   Avoid proteins, peptides, large MW drugs
2. **Polar or Charged Drug**
   LogD 0-2 recommended;
   Need appreciable neutral fraction
3. **High Plasma Protein Binding**
   Free fraction >0.10 recommended
4. **Active Efflux Transport**
   Avoid drugs with affinity for P-gp, MRP, BCRP, etc…

Her-2 Status and the Development of Brain Metastases

Of 122 women receiving Herceptin™ +/- chemotherapy, symptomatic CNS metastases were identified in 34%.
Fifty percent of the patients were responding to therapy, or had stable disease when they developed CNS metastases.

Cancer 97: 2972, 2003

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

CNS Events in Trastuzumab Adjuvant Trials

	NSABP B-31	N9831	HERA
Median follow-up (yr)	2.4	1.5	1.0
Any distant metastasis as first event: No (%)			
1 year trastuzumab	60 (6.9%)	30 (3.7%)	85 (5.0%)
No trastuzumab	111 (12.7%)	63 (7.8%)	154 (9.1%)
CNS mets as a first event: No events			
1 year trastuzumab	21	12	21
No trastuzumab	11	4	15
CNS mets at any time: No. events			
1 year trastuzumab	28	Not Reported	Not Reported
No trastuzumab	35 (P=0.35)		

Clin. Cancer Res. 13:1648, 2007

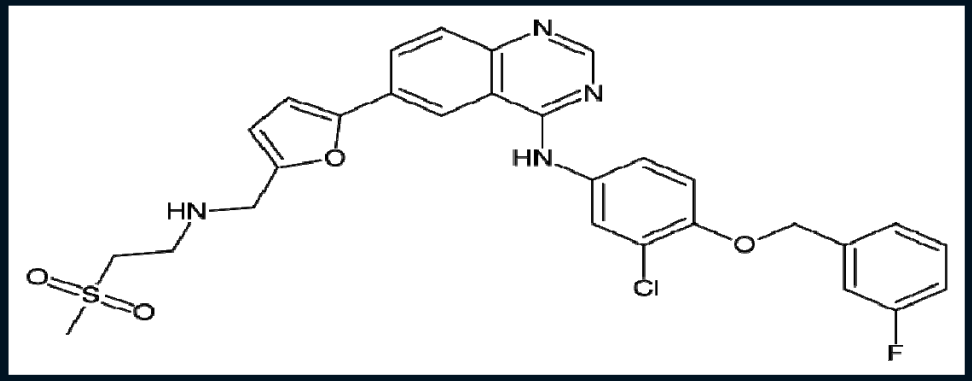
Her-2 Overexpression Promotes the Metastatic Colonization of the Brain by Breast Cancer

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**

Cancer Res. 67:4190, 2007

Lapatinib

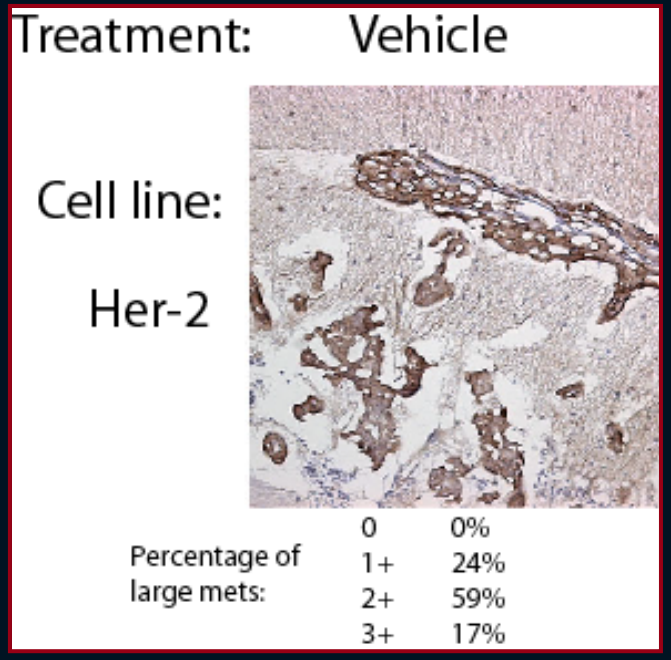
- Dual tyrosine kinase receptor, Her2 and EGFR1 inhibitor.
- High selectivity and affinity (K_d = 10 nM)
- Binds to ATP site of tyrosine kinase domain
- Non-polar lipophilic molecule
  - Log P value > 4.6 MW : 581 Daltons
  - P-GP & BCRP substrate
- IC_{50} ranging from 0.01 - 18 µM against a series of breast cancer cell lines.

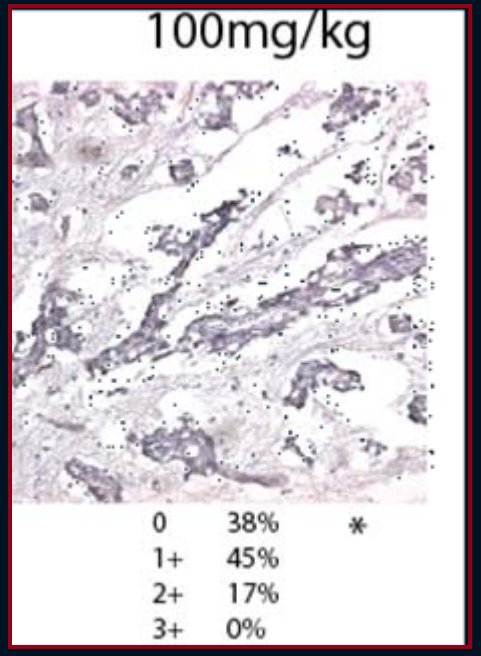


Lapatinib Prevents Brain Metastatic Colonization

Cell Line:	Treatment:	N	Mean (95% Confidence Interval) Metastases: Micrometastases:	Clinical 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**





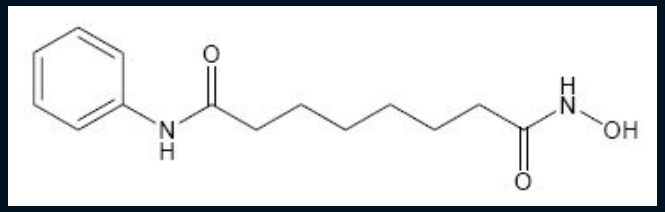
- **Only prevented colonization of 231-BR cells (EGFR+) cells at highest dose tested**
- **No interaction with radiation in Her-2+ cells.**

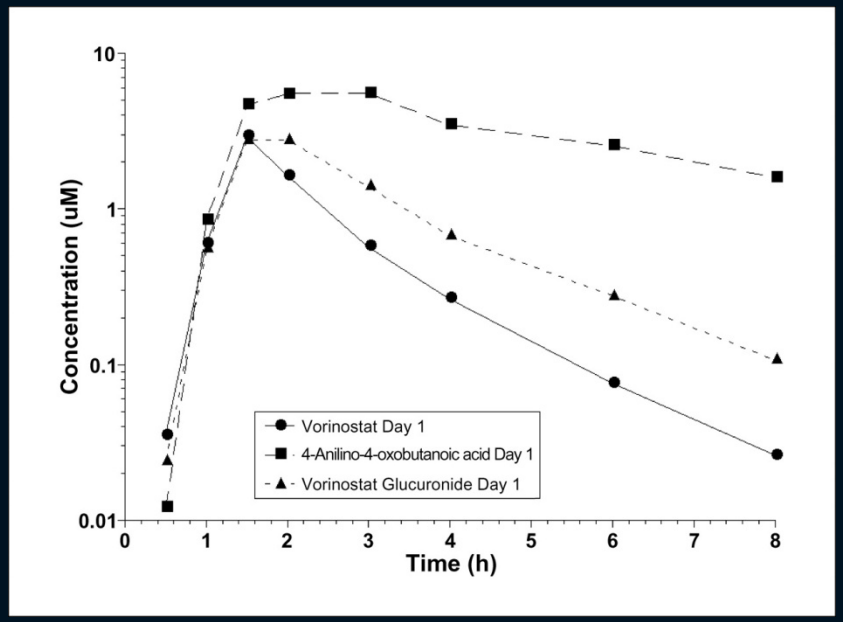
JNCI 100(15):1092, 2008

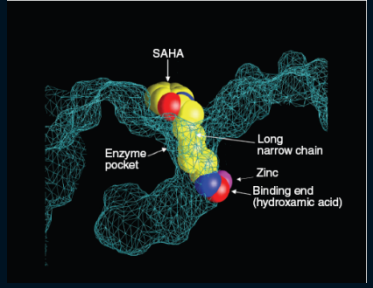
SAHA – Vorinostat

Histone Deacetylase Inhibitor

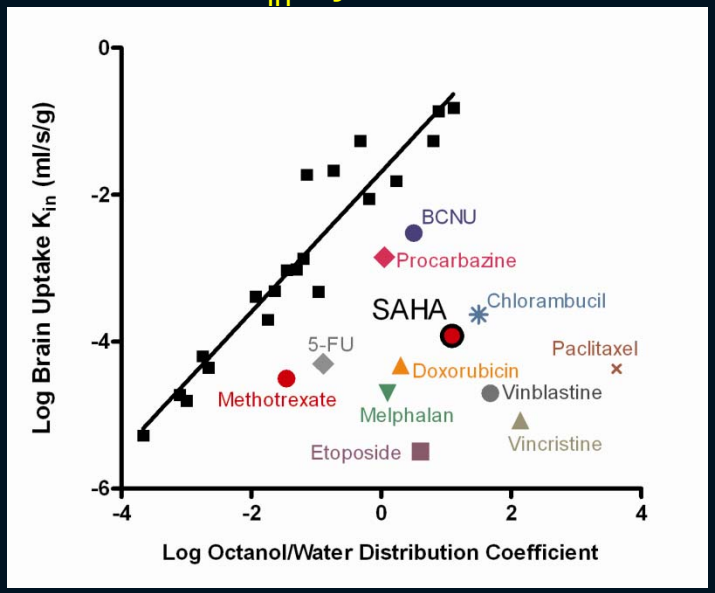
- MW: 264 H Bond Donors: 3
- LogD: 1.09 H Bond Acceptors: 5
- Plasma f_u: 0.29 (71% Bound)

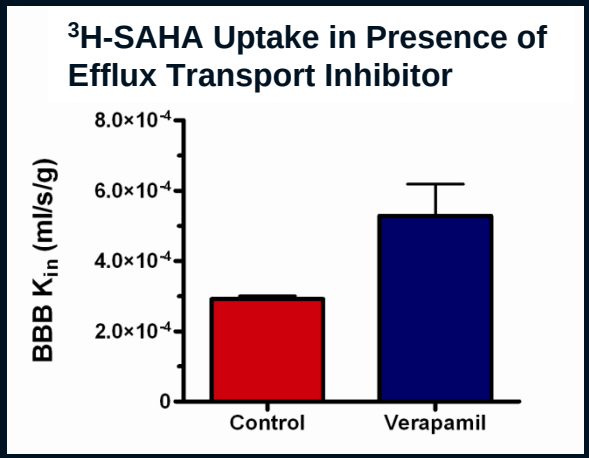






SAHA BBB K_{in} by Brain Perfusion

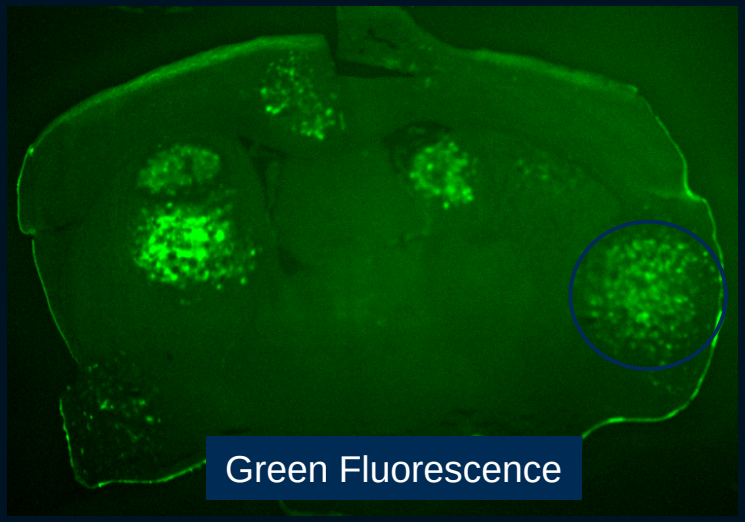


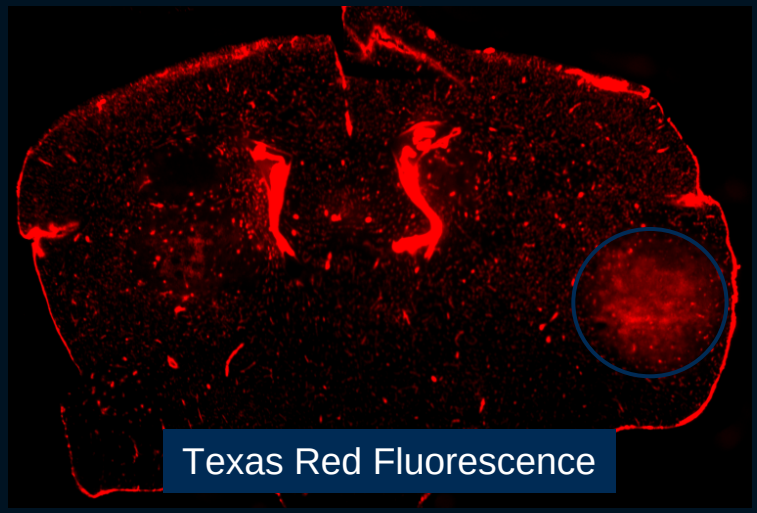


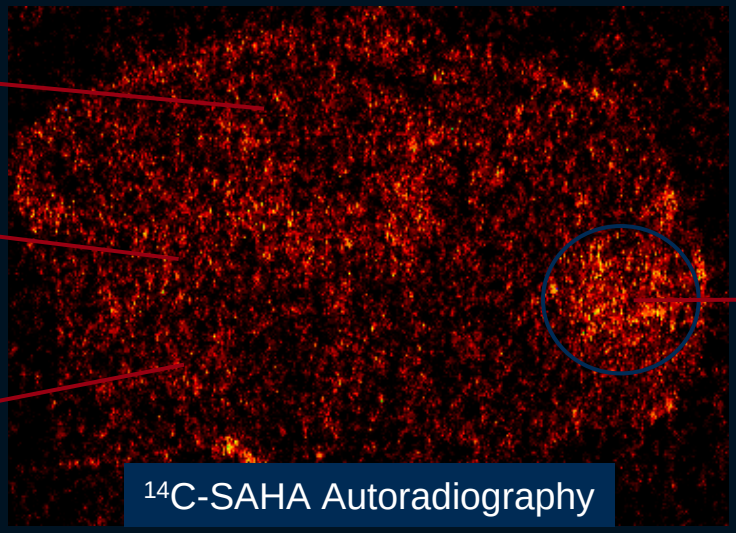
^{14}C-SAHA Distribution in Brain Metastases

(Brain/Blood = 7.5 ±1.1%)

Intracardiac MDA-MB-231BR High Her2 – 4 wk; 150 mg/kg (2.5 µCi) ^{14}C-SAHA iv – 30 min circulation





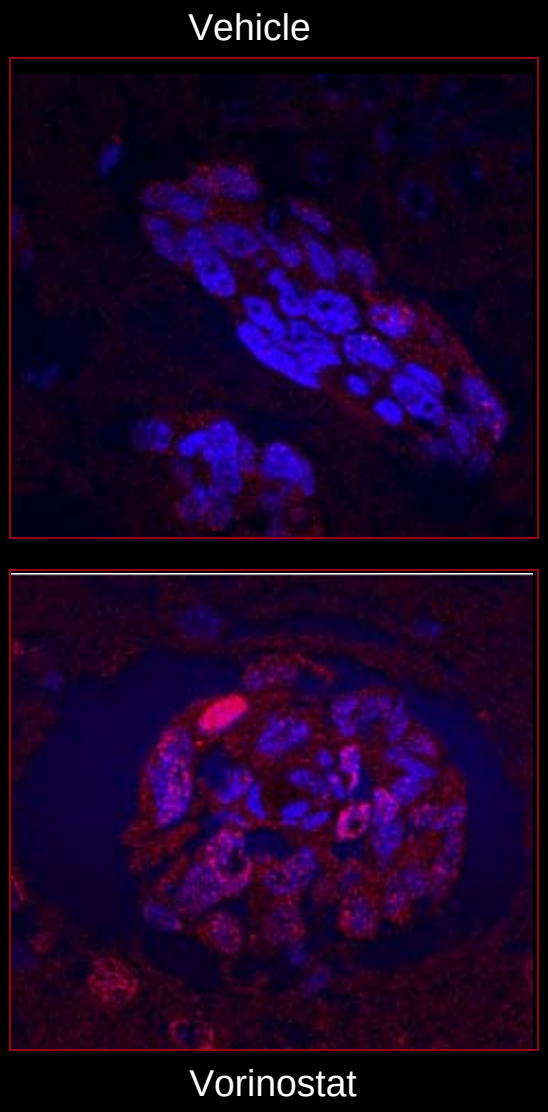


Vorinostat (SAHA) Prevents the Development of Experimental Brain Metastases When Administered Early

Treatment	N	Micrometastases:			Clinical Metastases:		
		Mean[2]	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

Studies on the In Vivo Mechanism of Action of Vorinostat

Mechanism:	In Vitro:	In Vivo:
Increased Histone Acetylation	+	No
Reduced Proliferation	+	Trend Only
Apoptosis	+	No
DNA Double Strand Breaks	+	+
Altered Gene Expression	↑ ↓	↓ Hes1 Rad52



Lessons Learned…

- It will be easier to prevent brain metastases than to cure an established lesion. How should these trials be designed?

- Synergy with radiation may be key to therapy trials. (Vorinostat?)

- There are therapeutic targets for brain metastases that we have not yet explored. They may not all be obvious from primary tumor data.

- Brain permeable drugs are needed. These may come in at least two flavors-
  - Permeable in normal brain (Vorinostat)
  - Able to get into a subset of permeable mets (Laptinib?)

Normal brain permeability may be best for penetration of micrometastases/dormant cells, and to hold them in check?

- New technologies for overcoming the blood-brain barrier should receive high priority.

BrainMetsBC.org

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

Musa Mayer
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