Brain Metastasis of Breast Cancer: Molecular and Preclinical Advances

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Center for Cancer Research
National Cancer Institute
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
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

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

Fold increases in Texas Red 3kD fluorescence over BDT plotted versus tumor size

Quentin Smith Ph.D., Paul Lockman Ph.D., Texas Tech
Lesson 3: Heterogeneous and Distinct Patterns of Drug Uptake are Observed in Experimental Metastases

14C-Paclitaxel Distribution

14C-Doxorubicin Distribution

Fold Increase in 14C Concentration (Brain =1.0)

Presurgical Study Open at Cleveland Clinic to Confirm in Human Tumors

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

Kenneth Aldape
Department of Neurosurgery, MDACC

Cancer Res. 67:4190, 2007
Her-2 Transfectants of the 231-BR Cell Line

<table>
<thead>
<tr>
<th>p-Her-2</th>
<th>Her-2</th>
<th>p-Akt</th>
<th>Akt</th>
<th>p-EGFR</th>
<th>EGFR</th>
<th>p-ERK 1/2</th>
<th>Erk 1/2</th>
<th>p21</th>
<th>α-Tubulin</th>
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<tbody>
<tr>
<td>S</td>
<td>E</td>
<td>H</td>
<td>S</td>
<td>E</td>
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<tr>
<td>Vector-1</td>
<td>Low-1</td>
<td>High-1</td>
<td>Vector-2</td>
<td>Low-2</td>
<td>High-2</td>
<td></td>
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</tbody>
</table>

Increased pHer-2 pAkt p21

Increased Colonization Equivalent Proliferation, Endothelial invasion.

Cancer Res. 67:4190, 2007
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.
14C-Lapatinib Distribution in Brain Metastases

Green Fluorescence – GFP Tumor

Red Fluorescence – Texas Red 3kD Dextran

Texas Red Leakage

14C Autoradiography - Lapatinib

<table>
<thead>
<tr>
<th></th>
<th>2 hr</th>
<th>12 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (µM)</td>
<td>5.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Brain (µmol/kg)</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Brain metastases (µmol/kg)</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Systemic Metastases (µmol/kg)</td>
<td>----</td>
<td>3.4</td>
</tr>
</tbody>
</table>
# Lapatinib Inhibits Brain Metastatic Colonization

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

*** P < 0.0001

![Graph showing treatment effects](image_url)

*JNCI* 100: 1092, 2008
Lapatinib for Treatment of Brain Mets- Phase II Trial Results:

1 PR/39 patients
Some stable disease noted


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).


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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Trend on Microarray</th>
<th>Mean (95% Confidence Interval)</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>BHC80</td>
<td>Down in BM</td>
<td>22.5 (11.7-33.3)</td>
<td>0.034</td>
</tr>
<tr>
<td>BMP1</td>
<td>Down in BM</td>
<td>2.6 (1.67-3.52)</td>
<td>0.014</td>
</tr>
<tr>
<td>HK2</td>
<td>Up in BM</td>
<td>1.0 (1.47-0.57)</td>
<td>0.097</td>
</tr>
<tr>
<td>LOXL1</td>
<td>Down in BM</td>
<td>3.83 (2.55-5.11)</td>
<td>0.062</td>
</tr>
<tr>
<td>PEDF</td>
<td>Down in BM</td>
<td>5.35 (0.71-11.41)</td>
<td>0.068</td>
</tr>
<tr>
<td>SIAH</td>
<td>Down in BM</td>
<td>1.01 (0.83-1.18)</td>
<td>0.056</td>
</tr>
<tr>
<td>SLIT3</td>
<td>Down in BM</td>
<td>1.12 (0.55-1.69)</td>
<td>0.087</td>
</tr>
<tr>
<td>STHMN3</td>
<td>Down in BM</td>
<td>0.56 (0.14-0.98)</td>
<td>0.048</td>
</tr>
<tr>
<td>TSPD2</td>
<td>Down in BM</td>
<td>0.13 (0.02-0.24)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Micrometastases:</th>
<th>Clinical Metastases:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean 95% CI P</td>
<td>Mean 95% CI P</td>
</tr>
<tr>
<td>Vehicle</td>
<td>20</td>
<td>170 146-193</td>
<td>7.65 6.20-9.10</td>
</tr>
<tr>
<td>SAHA D3 post-inj.</td>
<td>18</td>
<td>122 98-146 0.017</td>
<td>2.89 1.94-3.84 &lt; 0.0001</td>
</tr>
<tr>
<td>SAHA D7 post-inj.</td>
<td>19</td>
<td>151 127-176 NS³</td>
<td>4.94 3.90-5.98 0.008</td>
</tr>
<tr>
<td>SAHA D14 post-inj.</td>
<td>18</td>
<td>177 153-201 NS</td>
<td>5.96 4.69-7.22 NS</td>
</tr>
</tbody>
</table>

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

Vorinostat Induced Double Strand DNA Breaks In Vitro and In Vivo

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
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Kevin Camphausen
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Harvard
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RTOG
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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
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