What Every Neuropathologist Needs to Know: Part II:

Molecular Aspects of Metastatic Brain Tumors

Ronald L. Hamilton, M.D.
Associate Professor of Pathology

Division of Neuropathology
University of Pittsburgh

LEARNING OBJECTIVES:

Recall appropriate molecular testing for metastatic breast cancers

Recall appropriate molecular testing for metastatic melanomas

Recall appropriate molecular testing for metastatic lung cancers

Introduction:

A paradigm shift has occurred in the evolution of how we treat patients with brain metastases.

No longer relegated to the realm of palliation with an expectation of a rapid neurological decline and inevitable neurological demise, patients with brain metastases now have a myriad of aggressive treatment options available to them, resulting in a longer life expectancy and better quality of life.

With the use of markedly improved local control measures, patients are now often just as likely to succumb from their systemic disease, than from their brain tumor(s).

Introduction:

- The incidence rate of primary brain tumors is 6.6 per 100,000 and there are about **25,000** primary malignant brain tumors per year in US
- Brain metastases outnumber primary neoplasms.
- Because no national cancer registry documents brain metastases, the exact incidence is unknown: It has been estimated that **98,000 to 170,000** new cases are diagnosed in the United States each year.
- The frequency of metastatic brain tumors is rising due to longer survival after primary cancer diagnosis, which is a direct result of earlier detection and more effective treatment.

Introduction:

- The most common primary cancers metastasizing to the brain are
  - lung cancer (50%),
  - breast cancer (15%–20%),
  - unknown primary cancer (10%–15%),
  - melanoma (10%), and
  - colon cancer (5%).
- Although the highest numbers of brain metastases come from the lung, it has been documented that melanoma has the highest propensity of all malignant tumors to metastasize to the brain.
- Brain metastasis may be a presenting symptom of a systemic malignancy, most often lung cancer.

Molecular evaluation

Molecular subtyping of brain metastases and implications for therapy.

Renfrow JJ, Lesser GJ

Curr Treat Options Oncol.

2013 Dec;14(4):514-27

Metastatic breast carcinoma

Ductal and lobular types:

- Progesterone receptor (PR)
- Estrogen receptors (ER)
- Her2/Neu evaluation
  - immunostaining
  - in situ hybridization for amplification

Primary breast cancers

- **ER+/PR+:** 60%
- **ER+/PR-:** 13%
- **ER-/PR+:** 2%
- **ER-/PR-:** About 25% of breast cancers fit into this category.
- HEr2/neu : 20%
- Triple-negative: 20%

Breast cancer

Hoefnagel et al. Receptor Conversion in distant breast cancer metastases. Breast Cancer Research 2010, 12:R75

Conversion was mainly from positive in the primary tumor to negative in the metastases for ER and PR, while HER2 conversion occurred equally both ways.

PR conversion occurred significantly more often in liver, brain and gastro-intestinal metastases.

ER and PR assessment: H-scores

H-scores:

Estimate percentage of tumor cell nuclei with:

No staining (0), Light staining (1+), Moderate staining (2+), Dark staining (3+)

Multiply each one by the staining level and then sum.

Example #1:

100% of the tumor cells are 3+; the H-score = (100 *3) = 300.

Example #2:

50% are 1+ and 50% are 0; the H-score = (1*50) + (0 *50) = 50

TEST: Calculate H-score 20% are 0,
30% are 1+,
30% are 2+
20% are 3+

ER and PR assessment: H-scores

Estimate percentage of tumor cell nuclei with:

No staining (0), Light staining (1+), Moderate staining (2+), Dark staining (3+)

Multiply each one by the staining level and then sum.

TEST: Calculate H-score

20% are 0,
30% are 1+,
30% are 2+
20% are 3+

H-score = (20*0) + (30*1) + (30*2) + (20*3)

= 0+30+60+60

= 150.

ER and PR

Immunostaining is done according to the ASCO-CAP Guidelines for breast cancer.

A positive ER or PR tumor shows nuclear immunostaining at least 1% of the tumor cells.

Trastuzumab (Herceptin) -

- Trastuzumab (Herceptin) is a specially made antibody that targets HER2/neu-positive (HER2/neu+) cancer cells.
- Clinical trials in women with HER2/neu+ metastatic breast cancer have shown trastuzumab can shrink tumors and slow cancer growth when used alone or combined with chemotherapy.
- Trastuzumab is used as a first treatment for HER2/neu+ metastatic breast cancer as well as a treatment for HER2/neu+ metastatic cancer that has started to progress with chemotherapy.
- In some cases, HER2/neu+ tumors may spread to the brain. Because trastuzumab is not able to cross the blood-brain barrier, it is not used to treat brain metastases.
- - Susan G Komen foundation

Her2/Neu evaluation

Tech: 4B5 antibody clone is used as part of FDA approved Pathway on the Benchmark XT (Ventana, Tucson, AZ) and interpreted as follows:

Score 0 (negative) = No staining is observed or membrane staining in less than 10% of the tumor cells.

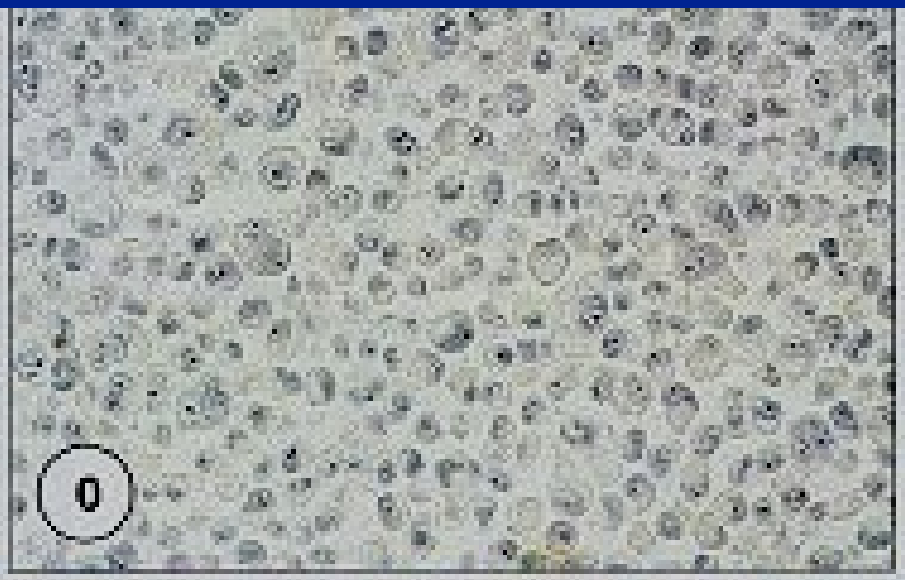
Score 1+ (negative) = Faint membrane staining in >10% of the tumor cells. The cells are only stained in part of their membrane.

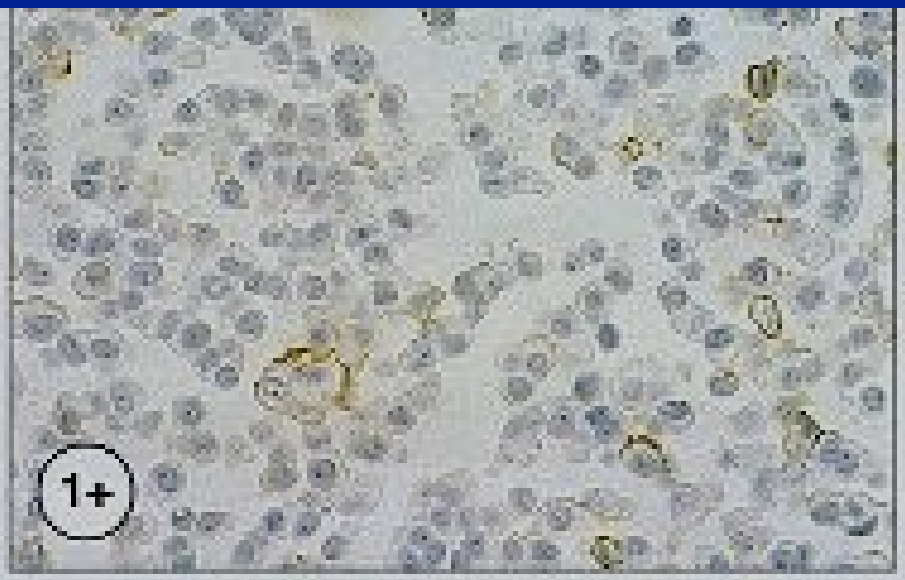
Score 2+ (equivocal) = A weak to moderate complete membrane staining is observed in **more than 10% of the tumor cells**.

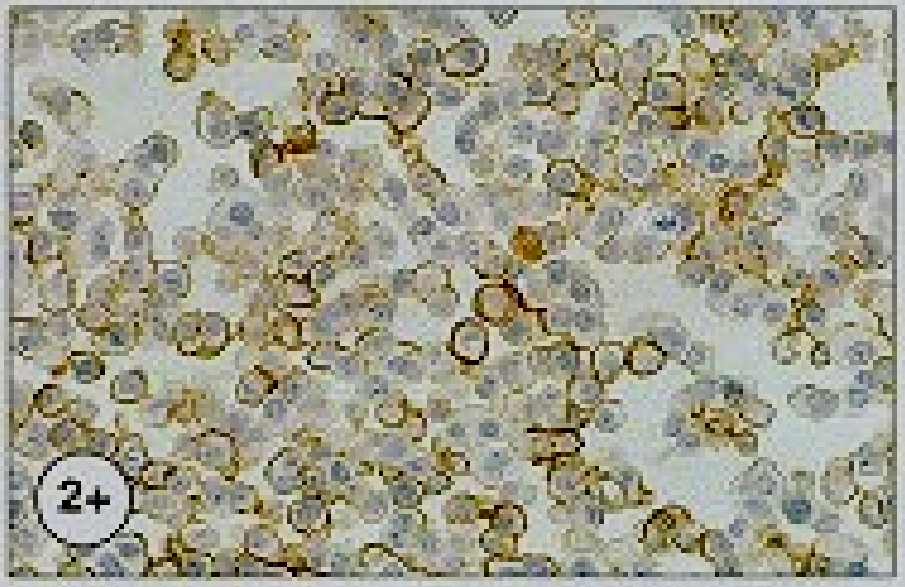
This score requires reflex testing by FISH.

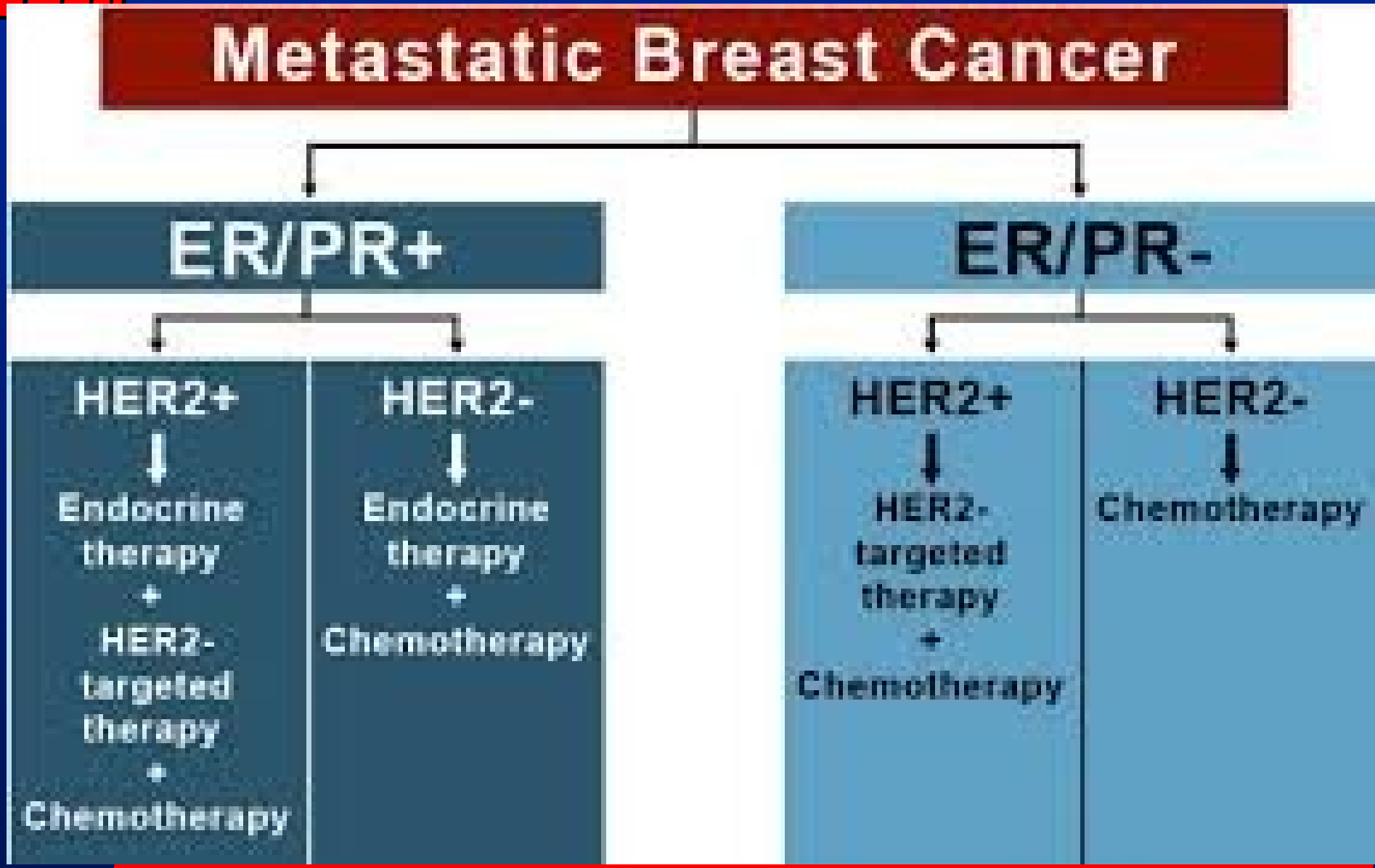
Score 3+ (positive) = A strong complete membrane staining is observed in more than **30% of the tumor cells.**

Metastatic breast








Metastatic Breast Cancer
ER/PR+
ER/PR-
HER2+
Endocrine therapy + HER2-targeted therapy + Chemotherapy
HER2-
Endocrine therapy + Chemotherapy
HER2+
HER2-targeted therapy + Chemotherapy
HER2-
Chemotherapy

Metastatic breast

ASCO-CAP guidelines for evaluation ER, PR or Her2/Neu in brain or bone metastases have not been validated in large studies

Metastatic melanoma

MUTATIONS:

NRAS

BRAF (V600E)

Metastatic melanoma

- Dabrafenib and vemurafenib are BRAF inhibitors that have demonstrated improved survival in patients with brain metastases from melanoma
- the use of these agents are the subject of several active clinical trials.

BRAF V600E immunostaining

Using DNA sequencing results as the reference, sensitivity and specificity for IHC were 98.2 % (55/56) and 98.1 % (413/421)

Day F1, Muranyi A, Singh S, Shanmugam K, Williams D, Byrne D, Pham K, Palmieri M, Tie J, Grogan T, Gibbs P, Sieber O, Waring P, Desai J. (2014) ***A mutant BRAF V600E-specific immunohistochemical assay: correlation with molecular mutation status and clinical outcome in colorectal cancer.*** *Target Oncol. 2014*

Long GV1, Wilmott JS, Capper D, Preusser M, Zhang YE, Thompson JF, Kefford RF, von Deimling A, Scolyer RA ***Immunohistochemistry is highly sensitive and specific for the detection of V600E BRAF mutation in melanoma.*** *Am J Surg Pathol. 2013 :61-5.*

Metastatic lung cancer NSCLC

FISH STUDIES:

1. **ALK rearrangement**

ALK inhibitor - LDK-378 (from Novartis)

2. **C-MET amplification**
3. **ROS1 rearrangement**
4. **KIF5B/RET translocation**

MUTATION STUDIES:

1. **PIK3CA**
2. **EGFR : exons 18 , 19, 20, 21**

erlotinib (Tarceva)

3. **BRAF : exon 15**
4. **KRAS:**

exon 2 (codons 12, 13) and exon 3 (codon 61)

NSCLC

- Phase II trials and retrospective reviews for gefitinib and erlotinib demonstrate these agents may have a role in both the chemoprevention of brain metastases and, in combination with WBRT, treatment for non-small cell lung cancer (NSCLC) brain metastases.

Summary:

1. Molecular subtyping of metastatic lung, breast and melanoma by neuropathologists is becoming increasingly important for clinical treatment decisions.
2. Molecular testing of metastases should be done in collaboration with the Hematology-Oncology team.