HER2 Immunostaining and FISH in Endometrial Serous Carcinoma

PRESENTED BY

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Natalia Buza reported no relevant financial relationships
PLEASE TURN OFF YOUR CELL PHONES
Preferred chemotherapy regimen for Her2-positive advanced stage (st III/IV) or recurrent endometrial serous carcinoma:

carboplatin/paclitaxel/trastuzumab
Endometrial Serous Carcinoma

~10% of endometrial cancer

Postmenopausal women, no association with hormones, obesity or hyperplasia

High histologic grade

Poor response to traditional chemotherapy

High rate of recurrence and mortality

~40% of endometrial cancer deaths
Adjuvant chemoradiotherapy versus radiotherapy alone in women with high-risk endometrial cancer (PORTEC-3): patterns of recurrence and post-hoc survival analysis of a randomised phase 3 trial

de Boer et al., Lancet Oncol 2019
Need for New Targeted Therapies

Interest in Her2 in Endometrial Cancer is not new…

1990s - 2010:

- Her2 overexpression 14-80%
- Her2 amplification 21-47%
  - Variation in testing and scoring methods and case inclusion criteria
- Case reports of successful Her2-based therapies
- GOG 181B clinical trial
Phase II trial of trastuzumab in women with advanced or recurrent, HER2-positive endometrial carcinoma: A Gynecologic Oncology Group study

GOG 181B

Single agent trastuzumab for advanced or recurrent Her2 positive endometrial cancer

33 patients with Her2 positive tumors: 11 serous, 13 endometrioid, 3 clear cell, 5 mixed, 1 NOS

FDA scoring criteria for breast cancer

IHC scores 2+ and 3+, or FISH ratio >2.0

No clinical activity; trial closed early due to poor accrual

Fleming et al., Gynecol Oncol, 2010
Randomized Phase II Trial of Carboplatin-Paclitaxel Versus Carboplatin-Paclitaxel-Trastuzumab in Uterine Serous Carcinomas That Overexpress Human Epidermal Growth Factor Receptor 2/neu

Amanda N. Fader, Dana M. Roque, Eric Siegel, Natalia Buza, Pei Hui, Osama Abdelghany, Setsuko K. Chambers, Angeles Alvarez Secord, Laura Havrilesky, David M. O’Malley, Floor Backes, Nicole Nevadunsky, Babak Edraki, Dirk Pikaart, William Lowery, Karim S. ElSahwi, Paul Celano, Stefania Bellone, Masoud Azodi, Babak Lituouhi, Elena Ratner, Dan-Arin Silasi, Peter E. Schwartz, and Alessandro D. Santin
Eligible participants from 11 study sites were assigned to a treatment (N = 61)

CP

Participants were assigned to the control (CP) arm (N = 29)

- Participant withdrew consent (n = 1)

Participants received CP (n = 28)

- Evaluable for PFS (n = 26)
  - CP cycles received
    - More than six (n = 2 [7%])
    - All six cycles (n = 21 [75%])
    - Fewer than six (n = 5 [18%])

  CP treatment outcomes:
  - Alive and progression free (n = 5)
  - Alive after progression (n = 11)
  - Dead after progression (n = 11)
  - Dead without progressing (n = 1)

CP + T

Participants were assigned to the experimental (CP + T) arm (n = 32)

- Participants were FISH negative at central pathology (n = 2)

Participants received CP + T (n = 30)

- Evaluable for PFS (n = 30)
  - CP + cycles received
    - More than six (n = 1 [3%])
    - All six cycles (n = 25 [83%])
    - Fewer than six (n = 4 [13%])

  CP + treatment outcomes:
  - Alive and progression free (n = 13)
  - Alive after progression (n = 6)
  - Dead after progression (n = 11)
  - Dead without progressing (n = 0)

Fig 1. CONSORT diagram. CP, carboplatin-paclitaxel; CP + T, carboplatin-paclitaxel plus trastuzumab; FISH, fluorescence in situ hybridization; PFS, progression-free survival.

Fader et al, J Clin Oncol 2018
A. Primary efficacy analysis:
One-sided log-rank $P = .0052$
HR, 0.44 (95% CI, 0.26 to 0.76)

B. Advanced disease:
One-sided log-rank $P = .013$
HR, 0.40 (95% CI, 0.20 to 0.80)

C. Recurrent disease:
One-sided log-rank $P = .0029$
HR, 0.14 (95% CI, 0.04 to 0.53)

Fader et al, J Clin Oncol 2018
Preferred chemotherapy regimen for Her2-positive advanced stage (st III/IV) or recurrent endometrial serous carcinoma:

carboplatin/paclitaxel/trastuzumab
1998: Trastuzumab approved for breast cancer
2000: Lapatinib approved for gastric cancer
2002: Pertuzumab approved for breast cancer
2004: GOG 181B trial (single-agent trastuzumab in endometrial cancer)
2006: Trastuzumab improves PFS and OS in endometrial serous carcinoma
2008: T-DM1 approved for breast cancer
2010: Trastuzumab biosimilars approved for breast and gastric cancer
2012: Lapatinib approved for breast cancer
2014: Pertuzumab approved for breast cancer
2016: Trastuzumab approved for breast and gastric cancer
2018: Trastuzumab approved for breast cancer
Evolution of Her2 Guidelines in Breast and Gastric Cancer

1998
- FDA/ Herceptest™ package insert
  - 3+ IHC: >10% strong complete

2007
- ASCO/CAP
  - 3+ IHC: >30% circumferential, strong, complete
  - FISH ratio >2.2

2008
- Pre-trial (ToGA) validation study
  - 3+ IHC: >10%, strong complete, basolateral or lateral
  - FISH ratio ≥ 2.0

2013
- ASCO/CAP
  - 3+ IHC: >10% circumferential, strong, complete
  - FISH ratio ≥ 2.0
  - FISH ratio <2.0 AND Her2 signal ≥6.0/nucleus

2016
- ASCO/CAP
  - 3+ IHC: >10%, strong complete, basolateral or lateral
  - FISH ratio ≥ 2.0, or <2 with >6 Her2 signal/nucleus

2018
- ASCO/CAP
  - 3+ IHC: >10% circumferential, strong, complete
  - FISH ratio ≥ 2.0
  - FISH ratio <2.0 AND Her2 signal ≥4.0/nucleus
## Her2 in Breast and Gastric Cancer

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<thead>
<tr>
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<th>Breast</th>
<th>Gastric</th>
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<tbody>
<tr>
<td><strong>Proportion Her2 positive</strong></td>
<td>15-25%</td>
<td>22%</td>
</tr>
<tr>
<td><strong>Heterogeneity IHC/FISH</strong></td>
<td>Rare</td>
<td>Common, 50%</td>
</tr>
<tr>
<td><strong>Basolateral/lateral IHC pattern</strong></td>
<td>Rare, 2+</td>
<td>Common, 3+</td>
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Toward standard HER2 testing of endometrial serous carcinoma: 4-year experience at a large academic center and recommendations for clinical practice

Natalia Buza¹, Diana

¹Department of Pathology, Obstetrics, Gynecology, a
CT, USA

Marked Heterogeneity of HER2/NEU Gene Amplification in Endometrial Serous Carcinoma

Natalia Buza and Pei Hui
Department of Pathology, Yale University School of Medicine, New Haven, CT, 06520-8023
108 cases (85 pure SC, 23 mixed SC)

**Her2 IHC** (DAKO Herceptest™)

**Her2 FISH** on all IHC 2+ cases, and in a smaller number of 0, 1+, and 3+ cases (PathVysion)

IHC scores per FDA and 2007 ASCO/CAP breast criteria

Her2 amplification: Her2/CEP17 ratio ≥ 2.0

Buza et al., Mod Pathol, 2013
Her2 in Serous Carcinoma

FDA (breast) criteria: 38% Her2 + (by either IHC/FISH)

ASCO/CAP 2007 (breast) criteria: 31% Her2 + (by either IHC/FISH)

Buza et al., Mod Pathol, 2013
IHC-FISH Concordance

All IHC cases:

- FDA: 75%
- ASCO/CAP 2007: 81%

IHC 2+ excluded:

- FDA: 78%
- ASCO/CAP 2007: 86%

Buza et al., Mod Pathol, 2013
# Her2 Immunohistochemistry

- **2007 ASCO/CAP breast guidelines:**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
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<tbody>
<tr>
<td>0</td>
<td>No staining in invasive tumor cells</td>
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<tr>
<td>1+</td>
<td>Weak, incomplete membrane staining in any proportion or weak, complete membrane staining in &lt;10% of tumor cells</td>
</tr>
<tr>
<td>2+</td>
<td>Complete membrane staining that is nonuniform or weak but with obvious circumferential distribution in at least 10% of cells or intense complete membrane staining in ≤ 30% of tumor cells</td>
</tr>
<tr>
<td>3+</td>
<td>Uniform intense membrane staining in &gt; 30% of tumor cells</td>
</tr>
</tbody>
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Wolff et al., Arch Pathol Lab Med, 2007
Lack of Apical Her2 Staining
Lack of Apical Her2 Staining
Marked Heterogeneity of Her2 Expression by IHC

31% of all serous carcinomas (33/108)

53% of Her2 positive tumors (20/38)

19% of Her2 negative tumors (13/70)
2007 ASCO/CAP breast guidelines:

Positive: Her2/CEP17 ratio > 2.2

Equivocal: Her2/CEP17 ratio 1.8 – 2.2

Patients with ratio ≥ 2.0 eligible for trastuzumab therapy

Wolff et al., Arch Pathol Lab Med, 2007
Heterogeneity of Her2 gene amplification

Cluster amplification in most cases with heterogeneous IHC

Mosaic amplification

Her2 protein expression by IHC closely correlates at the cellular level with Her2 gene amplification

Chr 17 polysomy is rare

Buza et al., Genes, Chromosomes & Cancer, 2013
Cluster Amplification
2007 ASCO/CAP breast with specific modifications:

Complete circumferential staining not required, basolateral/lateral pattern also accepted

Large tumor section(s) selected for IHC (on hysterectomy)

FISH on 2+ IHC only, in direct correlation with IHC

Larger tumor area selected for FISH ($\geq 1 \text{ cm}^2$)

$\text{Her2/CEP17 ratio } \geq 2.0$
Integrated genomic characterization of endometrial carcinoma

The Cancer Genome Atlas Research Network*

*TCGA, Nature 2013
TMA with 69 uterine serous carcinomas

Her2 overexpression by IHC: 20% (2007), 40% (2013)

Her2 amplification by CISH: 13%

IHC-CISH concordance (positive cases only):

ASCO/CAP 2007 64% (9/14)
ASCO/CAP 2013 32% (9/28)
Intratumoral heterogeneity of ERBB2 in 67%
Her2 IHC and FISH Assays (for Breast Cancer)

IHC:
- HercepTest (A085; DAKO)
- Pathway anti-Her2 (4B5; Ventana)
- Bond Oracle (CB11; Leica)

FISH:
- PathVysion (Abott)
- HER2 FISH PharmDx (DAKO)

Dual ISH:
- Inform Her2 (Ventana)

LDTs:
- www.fda.gov
Remaining Practical Issues

Correlation with clinical response: IHC or FISH (or NGS)?

Clinical impact of:
- FDA vs ASCO/CAP 2007 vs 2013 vs 2018 guidelines
- Her2 heterogeneity

Sample selection
- Bx/curettage vs hysterectomy
- Primary vs metastasis

Specimen handling/Fixation time
Sample Selection

“Does Specimen Type Have an Impact on Her2 Status in Endometrial Serous Carcinoma?” Rottmann, D. et al., abstract number #1083, poster #211

Stowell-Orbison Award Poster Session, Monday, March 2, 9:30 AM

95% concordance rate of HER2 status between biopsy and hysterectomy
Sample Selection

Primary vs metastasis:

23% of tumors with discordant Her2 expression on TMA

Halle et al, 2018 Br J Cancer
Potential Pitfalls

Heterogeneity:
- Immunohistochemistry
- FISH

Membrane staining pattern: complete vs. basolateral

Rare cases of co-amplification

Her2 testing of mixed carcinomas
Future Directions - Her2 in Carcinosarcomas

Her2 positive (ASCO/CAP 2007): uterine 14%, tubo-ovarian 7%
All Her2 positive uterine tumors had serous or mixed ca component
Summary

~30% of endometrial serous carcinomas Her2 positive

Addition of trastuzumab to chemotherapy significantly improves progression-free survival

ASCO/CAP 2007 breast scoring criteria with modifications:

- Lack of apical membrane staining
- Heterogeneity
- FISH on 2+ cases
  - Large area in correlation with IHC
  - Ratio $\geq 2.0$
THANK YOU!