## Disclosure Information

I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2016:

**Name of the enterprise / Nature of the interest**

<table>
<thead>
<tr>
<th>Enterprise</th>
<th>Interest</th>
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<tr>
<td>Employment: Scientific Fellow, Ventana Medical Systems, Inc., A Member of Roche Group</td>
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HER2 intratumoral heterogeneity in breast cancer: Proposal of a new classification

Hiroaki Nitta, Zaibo Li, Jorge Reis-Filho, Christos Sotiriou, Marie Pierre Chenard, Soren Nielsen, Giuseppe Viale, Anna Sapino, and Tibor Tot
Current definition of breast cancer HER2 intratumoral heterogeneity in the literature

**HER2 intratumoral heterogeneity is that HER2 gene status differs between cells in a single tumor**

Hanna WM et al., Mod Pathol (2014) 27, 4-18

**HER2 genetic heterogeneity exists if there are more than 5% but less than 50% of tumor cells with a HER2/CEN17 ratio higher than 2.2**

Vance GH et al., Arch Pathol Lab (2009) 133, 611-612

**Prevalence of HER2 genetic heterogeneity was 5-40% based on HER2/CEN17 ratio**

Hanna WM et al., Mod Pathol (2014) 27, 4-18
Why is HER2 intratumoral heterogeneity important?

HER2 intratumoral heterogeneity could be a cause of discordant HER2 status between:
1) Tissue blocks of the same tumor
2) Core biopsies and surgical tissue samples
3) Primary tumors and metastases
4) Individual assessment results
5) HER2 immunohistochemistry and HER2 in situ hybridization assays
6) Individual foci of a multifocal cancer

HER2 intratumoral heterogeneity could be a cause of:
1) Limited response to HER2-targeted therapy
2) Relapse of cancer
3) Low patient survival rates
HER2 gene-protein assay (GPA): Concurrent analyses of HER2 gene and HER2 protein at individual cell level

Nitta et al., Diagn Pathol (2012) 30;7:60
Discovery of *HER2* gene amplified tumor cells without HER2 protein overexpression
Our definition of HER2 epigenetic heterogeneity

HER2 epigenetic heterogeneity indicates the presence of HER2 gene amplified tumor cells without HER2 protein overexpression.
Materials and Methods

Patient cases
124 HER2 positive breast cancer cases (HER2 gene amplification and/or HER2 protein overexpression) determined with both:
   1) Roche HER2 immunohistochemistry
   2) Abbott HER2 fluorescence in situ hybridization

HER2 GPA
   1) HER2 gene and HER2 protein status confirmation
   2) HER2 heterogeneity statuses
Concurrent *HER2* gene and HER2 protein analyses:
“Classic” HER2 negative
*HER2* gene negative & HER2 protein negative

Objective 10X  
Objective 60X  
Objective 60X
Concurrent *HER2* gene and HER2 protein analyses:
“Classic” HER2 positive
*HER2* gene positive & HER2 protein positive
Concurrent *HER2* gene and HER2 protein analyses:

**Non-classic HER2 positive**

*HER2* gene positive & HER2 protein negative
Concurrent *HER2* gene and HER2 protein analyses:

**Non-classic HER2 negative**

Genetic heterogeneity - Isolated
Concurrent HER2 gene and HER2 protein analyses:
HER2 positive
Genetic heterogeneity - Clustered

Objective 10X  
Objective 60X  
Objective 60X
Concurrent HER2 gene and HER2 protein analyses:

HER2 positive

Genetic heterogeneity – Mixed (scattered)
Concurrent *HER2* gene and HER2 protein analyses:

**HER2 positive**

Epigenetic heterogeneity - Clustered
Concurrent *HER2* gene and HER2 protein analyses:

**HER2 positive**

Epigenetic heterogeneity – Mixed (scattered)

Objective 10X  
Objective 60X  
Objective 60X
Distribution of HER2 genetic heterogeneity and epigenetic heterogeneity among 124 HER2 positive breast cancer cases

- **Genetic heterogeneity**: 11.3% (14/124)
- **Epigenetic heterogeneity**: 29.8% (37/124)
Distribution of HER2 homogeneity and heterogeneity among 124 HER2 positive breast cancer cases

- **Homogeneity**: 60.5% (75/124)
- **Genetic heterogeneity (GH)**: 11.3% (14/124)
- **Epigenetic heterogeneity (EH)**: 29.8% (37/124)
- **Amplified HER2 gene tumor cells without HER2 protein “non-classic” HER2 positive**: 2.4% (3/124)
- **Isolated amplified HER2 gene tumor cells without HER2 protein “non-classic” HER2 negative**: 0.8% (1/124)
Proposed new breast HER2 heterogeneity types

- HER2 negative
  - Homogeneity
  - Heterogeneity

- HER2 positive
  - Clustering
  - Mixed
  - Genetic heterogeneity
  - Epigenetic heterogeneity
Conclusions

We propose a new classification of HER2 intratumoral heterogeneity that, in addition to HER2 genetic heterogeneity, includes a new HER2 epigenetic heterogeneity category.