

### Introduction

While HER2 testing is well established in directing appropriate treatment for breast cancer, a small percentage of cases show equivocal results by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). Alternative probes may be used in equivocal cases. We present the experience of a single community-based institution in further evaluating these cases.

# Methods

Between 2014 and 2016, 4255 samples were submitted for HER2 amplification testing by alternative probes, TP53, RAI1, and RARA. Of the patients tested by FISH, 12.9% also had IHC data.

# **Key Points**

- Some breast cancer cases tested for HER2 are equivocal by both IHC and FISH.
- The prevalence of double HER2 equivocal cases and the **Discrepancy between IHC and Alternative FISH Testing** discrepancy between IHC and alternative FISH testing 44% of IHC- cases became FISH+ by alternative probe (P<0.0001). suggest that FISH alternative testing using both RAI1 and 36% of IHC+ cases became FISH- by alternative testing (P<0.0001). TP53 probes is necessary for conclusive classification. • 52.3% IHC equivocal and 50.5% of FISH equivocal cases became HER2+ by alternative FISH.
- IHC repeat testing performed on different blocks showed **IHC Score 0 IHC Score 1** poor reproducibility. Negative Negative Alternative FISH No. HER2 amplified upon alternative testing, clinical studies to Negative 63.6 54.5 54 178 determine benefit of anti-HER2 therapy in these patients is Positive 45.5 36.4 45 urgently needed. Total 100.0 100.0
- Because almost half of FISH equivocal cases converted to

# Immunohistochemistry and Alternative FISH Testing in **Breast Cancer with HER2 Equivocal Amplification**

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# Results

- 73.9% of FISH equivocal cases remained equivocal after IHC testing.
- Upon duplicate testing by IHC using a different paraffin block, 44% of IHC- became equivocal and 40% of IHC equivocal became IHC-.

#### **FFPE Samples Available for Testing**

### **Repeat Testing by IHC**



No.	2 <sup>nd</sup> IHC Score	No.	%	P-Value	
4	0	1	25.0	NA	
	1	3	75.0		
32	0	2	6.3	P=0.001	
	1	16	50.0		
	2	14	43.8		
57	0	3	5.3	P=0.001	
	1	20	35.1		
	2	30	52.6		
	3	4	7.0		
3	2	3	100.0	NA	

HC Score 2 Equivocal		IHC Score 3 Positive		
	%	No.	%	
	47.7	4	36.4	
	52.3	7	63.6	
	100.0	11	100.0	

#### **Alternative Probes**

- 78% of positive cases were positive by RAI1 and 73.9% by TP53.
- 38% of all cases were deemed positive based on RAI1 probe, 36% on TP53, and 5% on RARA.



**Comparison between Probes Results in Duplicate Testing** 

Measured Probe	Reference Probe	Z
RAI1	TP53	2.02
RAI1	RARA	6.88
RAI1	RAI1	0.54
RARA	TP53	8.17
RARA	RARA	0.09

### HER2 Status Grouped by Drug Class

• 41% of FISH+ by alternative probe patients were treated with palbociclib.



• Most cases were positive by more than one probe.



Measured Probe	Reference Probe	Ζ	p-value
<b>TP53</b>	TP53	0.50	0.62
<b>TP53</b>	RARA	8.27	<0.00001
<i>CEP17</i>	<i>CEP17</i>	0.57	0.57
HER2	HER2	0.73	0.46
<i>CEP17</i>	D17Z1	5.60	<0.00001
	Measured ProbeTP53TP53CEP17HER2CEP17	Measured ProbeReference ProbeTP53TP53TP53RARACEP17CEP17HER2HER2D17Z1	Measured ProbeReference ProbeZTP53TP530.50TP53RARA8.27CEP17CEP170.57HER2HER20.73CEP17D17Z15.60

