Quantitative Measurement of Human Epidermal Growth Factor Receptor-2 (HER2) Protein Expression in 'Classical' and 'Non-classical' FISH Categories: a Comparative Study

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Background

HER2 FISH testing results in breast cancer can be categorized based on the HER2/CEP17 ratio and HER2 gene copy number according to the ASCO/CAP guideline. Cases with both a HER2/CEP17 ratio ≥ 2 and HER2 gene copy number ≥ 6 are considered 'classical amplified' (group 1 and 2). The HER2 FISH amplified cases with only *either* HER2/CEP17 ratio ≥ 2 *or* HER2 gene copy number ≥ 6 are considered 'non-classical' amplified such as 'low-level amplified' (group 3), "monosomy" (group 4), 'polysomy ratio negative' (group 5) (Fig 3). Press et al., has reported that 'non-classical' FISH amplified patients did not appear to have the same benefit from targeted therapy in terms of DFS or OS as 'classical' amplified patients. In the current study, we attempt to encompass all possible differentiable FISH categories that could be considered a unique subset. We expanded the HER2 FISH categories by adding 'polysomy ratio positive' (group 2) (subgroup the "classical amplified" cases with CEP17 > 2.7) (Table 2, Fig 3). We then used streptavidin coated Phosphor-integrated dot fluorescent nanoparticles (PID), a novel quantitative methodology, to measure HER2 protein receptor expression in each HER2 FISH category. We looked for the PID protein expression levels in different FISH categories. We compared the 'non-classical' FISH amplified categories to 'classical' amplified and 'classical' non-amplified categories to determine which categories were comparable (Table 2, Fig 1, 3).

Materials and Methods

159 invasive breast cancer cases were retrospectively selected from the University of Rochester Medical Center's (URMC) FISH database based upon the differing HER2 FISH results (Table 1). The cases had previously undergone IHC and FISH analysis. PID staining and quantitation was completed on all 159 cases for both PID/Cell and PID/ROI100μm^2.

Parameter	n (Average)	% (Range)	Parameter	n (Average)	% (Range)
N Total	159		ER/PR Status		
Age	(63.4)	(27-89+)	ER+/PR+	105	66.9
Diagnosis (DX)			ER+/PR-	17	10.8
IDC	145	91.2	ER-/PR+	3	1.9
ILC	12	7.5	ER-/PR-	32	20.4
Mixed	2	1.3	HER2 IHC Score		
Nuclear Grade			0	15	9.4
1	7	4.8	1+	19	11.9
2	52	35.9	2+	102	64.2
3	86	59.3	3+	23	14.5
ER Allred Score			Tumor Size (cm)		
0-2 (-)	37	22.2	> 5	16	12.8
3-8 (+)	130	77.8	2-5	64	51.2
PR Allred Score			1-2	35	28.0
0-2 (-)	51	30.5	< 1	10	8.0
3-8 (+)	116	69.5			

Table 1: Patient demographics and pathologic feature (N = 159)

	(Group #) Study FISH Categories	N	Mean Age	Nuclear Grade (%) 1	2	3	ER+ (%)	PR+ (%)	IHC Score (%) 0-1+	2+	3+
(1)	Classic Amplified	22	61.2	0 (0.0)	6 (30.0)	14 (70.0)	14 (63.6)	9 (40.9)	0 (0.0)	9 (40.9)	13 (59.1)
(2)	Polysomy Pos. Ratio	29	65.7	1 (3.7)	3 (11.1)	23 (85.2)	23 (79.3)	20 (69.0)	3 (10.3)	18 (62.1)	8 (27.6)
(3)	Low-Level Amplified	27	65.2	0 (0.0)	7 (30.4)	16 (69.6)	19 (73.1)	19 (73.1)	3 (11.1)	24 (88.9)	0 (0.0)
(4)	Monosomy	9	53.8	2 (25.0)	4 (50.0)	2 (25.0)	9 (100.0)	7 (77.8)	1 (11.1)	8 (88.9)	0 (0.0)
(5)	Polysomy Neg. Ratio	20	63.8	0 (0.0)	3 (17.6)	14 (82.4)	17 (85.0)	16 (80.0)	0 (0.0)	19 (95.0)	1 (5.0)
(6)	Equivocal	23	65.7	1 (4.8)	12 (57.1)	8 (38.1)	18 (81.9)	18 (81.9)	1 (4.3)	22 (95.7)	0 (0.0)
(7)	Classic Non-Amplified	29	62.1	3 (10.3)	17 (58.6)	9 (31.0)	22 (75.9)	19 (65.5)	26 (89.7)	2 (6.9)	1 (3.4)

Table 2: Patient pathologic characteristics broken down in FISH categories.

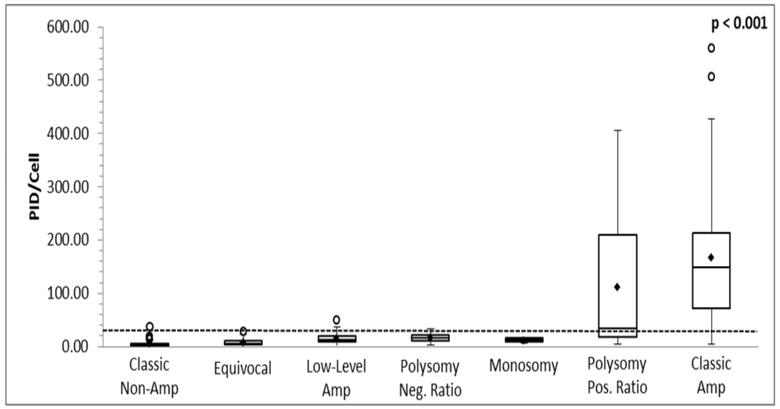

Figure 1 : HER2 protein expression in different FISH HER2 categories measured by PIDs

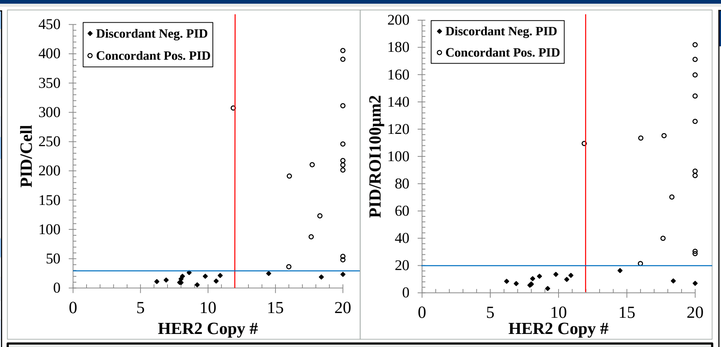

Figure 2: Discordant and concordant cases in "polysomy ratio positive' category (group 2)

	Polysomy Ratio Negative (Group 5)	Polysomy Ratio Positive (Group 2) Low HER2 PID (Discordant cases)	High HER2 PID (Concordant cases)
N	20	14	15
PID score @cell	16.0 (SD 7.5)	16.6 (SD 6.3)	202.6 (SD 50.3)
HER2 copy number (SD)	7.1 (1.8)	10.5 (4.5)	18.5 (SD 2.3)
CEP17 copy number	4.7	3.3	3.8
Ratio (SD)	1.5 (0.2)	3.3 (1.5)	5.3 (1.7)
Tumor size	2.7	2.8	3.7
Tumor grade	3.0	2.8	2.8
IHC 0-1 (%)	0 (0.0)	3 (21.4)	0 (0.0)
IHC 2 (%)	19 (95.0)	11 (78.6)	7 (46.7)
IHC 3 (%)	1 (5.0)	0 (0.0)	8 (53.3)

Table 3: The Patient pathologic characteristics in FISH HER2 categories with polysomy (Group 2 and 5). The discordant and concordant cases in group 2 can be sub-grouped.

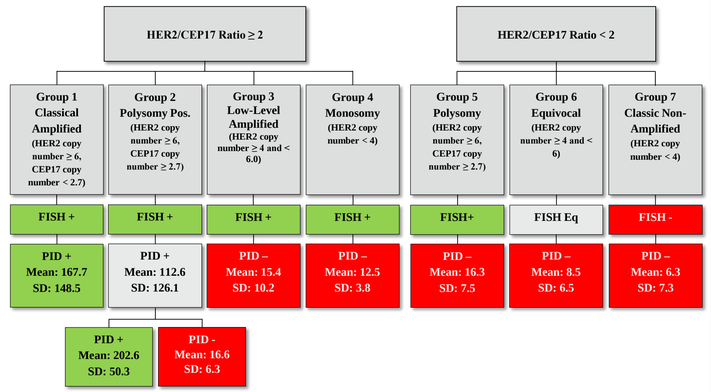

Figure 3: Flow diagram of different FISH categories and corresponding PID results

Results

The 'non-classical' amplified (groups 3, 4, 5) and equivocal categories (groups 6) were all found to have low-levels of protein expression similar to group 7 ('classical' non-amplified) categories. Their HER2 protein expression PID scores are below the experimental threshold values, which were predictive of a lower likelihood of a good pathologic response (RCB 0 or 1) to Trastuzumab based neoadjuvant therapy proposed in the previous study. (Figure 1).

The "polysomy ratio positive' category (group 2) showed that HER2 protein expression PID scores of this group are comparable to the "classical" amplified categories with significant variability (Fig 1 and 2). The discordant (low PID) 'polysomy ratio positive' cases showed distinct pathologic features such as HER2 IHC, average HER2/CEP17 ratio and average HER2 gene copy number that were comparable to 'polysomy ratio negative' cases (Table 3, Fig 3).

Conclusions

'Non-classical' FISH amplified categories (group 3, 4 , 5) and equivocal category (group 6) were all comparable to the 'classical' non-amplified FISH category when measured by PID. This suggests that these 'non-classical' FISH amplified and equivocal categories may be less likely to respond to targeted HER2 therapy. The "polysomy ratio positive' category (group 2) examined in this study is considered "classical" amplified in the current ASCO/CAP guideline report and most clinical studies. Group 2 PID results showed a subgroup of cases that had low HER2 protein expression levels, which indicate that there are a small percentage of polysomy "classical" amplified breast cancer cases may be less likely to respond to HER2 targeted therapy. These controversial cases warrant follow up studies with a larger patient cohort and may be better categorized using both FISH and quantitative HER2 protein analysis such as PID.

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1 - The authors of this abstract have indicated that they have no conflicts of interest that relate to the content of this abstract.
2 - The authors of this abstract have indicated the following conflicts of interest that relate to the content of this abstract: Dr. Hideki Goda, Dr. Takako Natori, Hisatake Okada and Dr. Yasushi Nakano are employees of Konica Minolta.