EGFR L858R Antibody Cross-Reaction in HER2 Positive Breast and Gastric Carcinomas

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BACKGROUND

The use of epidermal growth factor receptor (EGFR) mutation-specific antibodies is likely to get soon incorporated into clinical practice due to its proven correlation with the presence of EGFR mutation in lung adenocarcinoma, especially in cases with limited tumor material, or in situations where molecular genetic analysis is not readily available.

The use of these antibodies has also been suggested as an additional tool for distinguishing primary versus metastatic carcinomas in the lung. Incidental findings in our routine practice and a recently published paper reporting false positive breast carcinomas for EGFR L858R, made us think about the possible existence of a cross-reaction between HER2 and the EGFR L858R-specific antibody.

Aims of this study were to further analyze the existence of this cross-reaction and its frequency in the two common sources of metastatic tumors to lung, breast and gastric cancer; in which, in addition, HER2 expression is widely studied in relation to targeted therapies.

DESIGN

The series consists of 55 primary tumors, 22 breast and 5 gastric carcinomas HER2 positive for overexpression and amplification, and 20 breast and 8 gastric carcinomas negative for both (Tables 1 and 2).

Breast carcinomas (n=42)	
Age, mean (range), years	61 (37-89)
Tumor size, mean (range), cm	1.9 (0.1-7.5)
Histological subtypes	
IDC	33 (79%)
ILC, classical	3 (7%)
ILC, pleomorphic	4 (10%)
Mixed ductal and lobular	1 (2%)
DCIS	1 (2%)
ER, PR, HER2 status	
ER/PR +, HER2 –	19
ER/PR +, HER2 +	13
ER/PR –, HER2 +	9
ER/PR –, HER2 –	1

Table 1. Clinicopathological characteristics of patients with breast carcinoma.
Abbreviations: IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; DCIS, ductal carcinoma in situ.

Gastric carcinomas (n=13)	
Age, mean (range), years	70 (35-83)
Type of sample	
Gastrectomy	6
Biopsy	7
Histological subtypes	
Intestinal	2 (15%)
Diffuse	8 (62%)
Undifferentiated	3 (23%)
HER2 status	
HER2 –	8
HER2 +	5

Table 2. Clinicopathological characteristics of patients with gastric carcinoma.

Cases were assigned a score based on the ASCO/CAP guideline recommendations for HER2 testing in breast cancer and ToGA HER2 score in gastric cancer.

EGFR mutations were studied by immunohistochemistry with two specific monoclonal antibodies (EGFR [E746_750del] clone 6B6 and EGFR L858R clone 43B2) in all cases, and confirmed using real-time PCR when positive.

IHC delE746_A750 (exon 19)	**HER2 positive**	**HER2 negative**	**p-value**
			1.000
Positive	0	0	
Equivocal	0	0	
Negative	22 (100%)	20 (100%)	
IHC L858R (exon 21)	**HER2 positive**	**HER2 negative**	**p-value**
			$1.946x10^{-12}$
Positive	19 (86%)	0	
Equivocal	3 (14%)	0	
Negative	0	20 (100%)	

Table 3. Results of IHC staining with exon 19 EGFR [E746_750del] and exon 21 EGFR L858R mutation-specific antibodies and HER2 status on breast carcinoma cases.

IHC delE746_A750 (exon 19)	**HER2 positive**	**HER2 negative**	**p-value**
			1.000
Positive	0	0	
Equivocal	0	0	
Negative	5 (100%)	8 (100%)	
IHC L858R (exon 21)	**HER2 positive**	**HER2 negative**	**p-value**
			$0.777x10^{-3}$
Positive	5* (100%)	0	
Equivocal	0	0	
Negative	0	8 (100%)	

Table 4. Results of IHC staining with exon 19 EGFR [E746_750del] and exon 21 EGFR L858R mutation-specific antibodies and HER2 status on gastric carcinoma cases. *Three out of five positive cases showed focal expression.

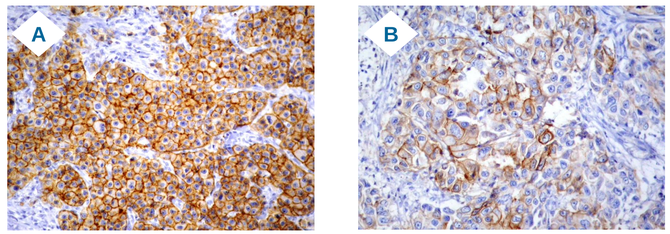

Figure 1. Example of HER2 positive breast cancer tumor (A) and HER2 positive gastric cancer tumor (B), both with IHC 3+ and HER2/CEP17 ratio >2.2 by FISH, showing a positive IHC stain for EGFR L858R mutation-specific antibody (x200).

RESULTS

In our study 86% (19/22) of HER2 positive breast carcinomas and 100% (5/5) of HER2 positive gastric carcinomas showed EGFR L858R positive expression (Fig 1), and equivocal in 14% (3/22) of HER2 positive breast carcinomas, while no expression was found in none of the HER2 negative carcinomas (Tables 3 and 4).

All cases were negative using the EGFR exon19 [E746_750del] antibody. Real-time PCR did not confirm the presence of the EGFR L858R mutation on neither of the 24 positives nor of the 3 equivocal cases by IHC (Fig 2).

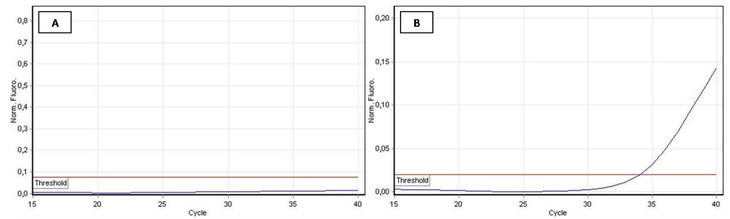

Figure 2. Therascreen results showing: (A) absence of L858R EGFR mutation and (B) valid internal control of the same reaction.

CONCLUSIONS

- EGFR L858R antibody gives false positive results in most of breast and gastric carcinomas with HER2 overexpression/amplification.
- The existence of this cross-reaction makes necessary verify any EGFR L858R positivity by molecular methods and discard the presence of HER2 overexpression/amplification in order to establish its clinical significance.
- HER2 has been described in other carcinomas, including lung cancer, although infrequently. In any case, is essential stressing the importance of achieving an accurate diagnosis, prior to use this antibody.

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