Letter

FISH and immunohistochemical status of the hepatocyte growth factor receptor (c-Met) in 184 invasive breast tumors

Alma Carracedo1,2,3, Kristof Egervari4, Marta Salido1,3, Federico Rojo5,6,7, Josep M Corominas5, Montserrat Arumí5,6,8, Cristina Corzo9, Ignacio Tusquets10, Blanca Espinet1,3, Ana Rovira6, Joan Albanell6,10, Zoltan Szollosi4, Sergi Serrano5 and Francesc Solé1,3

1Servei de Patologia, Laboratori de Citogenètica Molecular, Hospital del Mar, IMAS, GRETNHE, IMIM, 08003 Barcelona, Spain
2Departament de Biologia Cel·lular, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
3Escola de Citologia Hematològica S Woessner-IMAS, 08003 Barcelona, Spain
4Department of Pathology, University of Debrecen MHSC, 4032 Debrecen, Hungary
5Servei de Patologia, Unitat de Patologia Mamària, Hospital del Mar, UAB, 08003 Barcelona, Spain
6Molecular Therapeutics and Biomarkers in Breast Cancer Program, IMIM-Hospital del Mar, 08003 Barcelona, Spain
7Capio-Fundación Jiménez Díaz, 28040 Madrid, Spain
8Department of Health and Experimental Sciences, Universitat Pompeu Fabra, 08003 Barcelona, Spain
9Escola Bonanova-IMAS, 08003 Barcelona, Spain
10Oncology Department, Hospital del Mar, 08003 Barcelona, Spain

Corresponding author: Francesc Solé Ristol, F.Solé:Fsole@imas.imim.es

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In their report, Götte and coworkers [1] analyzed the expression of c-Met in 200 patients with ductal carcinoma in situ. They concluded that c-Met could be related to angiogenic and lymphangiogenic factors in ductal carcinoma in situ. On the other hand, Greenberg and coworkers [2] studied 31 patients with ductal infiltrating carcinoma (DIC) to detect c-Met expression in their axillary fluids. They observed a correlation of c-Met expression with increasing tumor size and grade, capillary and lymphatic invasion and lymph node metastasis.

We applied the fluorescent in situ hybridization (FISH) technique using the LSI D7S486/CEP7 commercial probe (Abbott Molecular Inc., Des Plaines, IL, USA), which includes the MET gene, and immunohistochemistry using c-Met monoclonal antibody clone 3D4 (Invitrogen, Carlsbad, CA, USA) to 184 archival invasive breast tumors (93 DIC and 91 lobular carcinomas). We constructed ten tissue microarrays with three replicates per sample. Pearson’s chi-squared and Fisher’s exact test were used to analyze the results.

None of the 155 breast tumors analyzed by FISH presented amplification of MET and 35 cases (22%) had a low grade of polysomy (three to five copies) of chromosome 7. Polysomy was more frequently observed in DIC (25%; $P = 0.001$). We tried to correlate polysomy of MET in the DIC group with grade, tumor size, lymph node status, clinical stage and expression of HER2, P53, estrogen receptor (ER) and progesterone receptor (PR). We observed that the absence of expression of PR was the unique statistically significant variable ($P = 0.001$). Moreover, the ER+/PR- samples presented the highest rate of polysomy (38%) compared to ER+/PR+ tumors (15%) (Table 1).

Out of 168 tumors analyzed by immunohistochemistry, 65 (38.7%) presented expression of c-Met. When histological types were compared, the DIC group also showed the highest number of c-Met-positive samples (48%; $P = 0.001$). From the analysis with the clinico-pathological variables, the negativity for PR was again statistically significant ($P = 0.001$). The ER+/PR- tumors presented more frequent expression of c-Met (68%) compared to ER+/PR+ tumors (32%) and were correlated with polysomy ($P = 0.020$) (Table 2).

We can conclude that amplification of MET in breast cancer is not a common event, as opposed to other cancer subtypes (renal, gastric and lung carcinomas). Although found in breast tumors, it seems that overexpression of c-Met is not mainly due to increased gene copy number of MET/polysomy7. However, polysomy in the ER+/PR- group could be an

DIC = ductal infiltrating carcinoma; ER = estrogen receptor; FISH = fluorescent in situ hybridization; PR = progesterone receptor.
important mechanism - although not the only one - responsible for the differential expression observed in this type of DIC. This c-Met overexpression and the presence of polysomy 7 could be important events to be considered with regard to the known poor response to endocrine therapies of ER+/PR- breast tumors. Lack of PR expression in ER+ tumors may be a surrogate marker of aberrant growth factor signaling [3] that could be associated with their more aggressive outcome, as has already been described [4].

Our study suggests that it would be interesting to investigate new therapeutic options for ER+/PR- DIC, which may include c-Met inhibitors.

### Competing interests
The authors declare that they have no competing interests.

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#### Table 1

| Carcinoma type | IHC c-Met | FISH MET | FISH + IHC |
|----------------|-----------|----------|------------|
|                | Negative  | Positive | Negative   | Polysomy | PE + P |
| Lobular        | 57 (76%)  | 18 (24%) | 61 (81%)   | 15 (19%) | 5 (7%) |
| Ductal (DIC)   | 42 (52%)  | 38 (48%) | 60 (75%)   | 20 (25%) | 13 (16%) |

| DIC type       | ER+/PR+   | ER+/PR- |
|----------------|-----------|---------|
| Lobular        | 31 (68%)  | 15 (32%)|
| ER+/PR+        | 31 (68%)  | 15 (32%)|
| ER+/PR-        | 11 (32%)  | 23 (68%)|
| Ductal (DIC)   | 42 (52%)  | 38 (48%)|
| ER+/PR+        | 39 (85%)  | 7 (15%) |
| ER+/PR-        | 21 (62%)  | 13 (38%)|

DIC, ductal infiltrating carcinoma; PE, positive expression; ER, estrogen receptor; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; P, polysomy; PR, progesterone receptor. In bold we remark the positive FISH and IHC results for DIC as well as for ER+/PR- tumors.

#### Table 2

| IHC and FISH results of MET according to the status of PR receptor in DIC carcinomas |
|-------------------------------------------|----------------|----------------|----------------|
| ER+/PR+ (n= 46)                          | FISH Negative | IHC Negative | IHC Positive |
|                                          | FISH Polyosmy | 27 (59%)      | 12 (26%)      |
|                                          |                | 9 (23%)       | 13 (38%)      |
| ER+/PR- (n = 34)                         | FISH Negative | 4 (9%)        | 3 (6%)        |
|                                          | FISH Polyosmy | 3 (9%)        | 10 (29%)      |

DIC, ductal infiltrating carcinoma; ER, estrogen receptor; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; P, polysomy; PR, progesterone receptor. In bold we remark the FISH and IHC positive results to compare both groups.

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