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# Outcome impact of *PIK3CA* mutations in HER2-positive breast cancer patients treated with trastuzumab

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**Background:** Phosphatidylinositol 3-kinase (PI3K) pathway activation has been suggested to negatively influence response to anti-HER2 therapy in breast cancer patients. The present study focused on mutations of the *PIK3CA* gene, encoding one of the two PI3K subunits.

**Methods:** *PIK3CA* mutations were assessed by direct sequencing in 80 HER2-positive patients treated with 1 year of trastuzumab. All patients preoperatively received four cycles of anthracycline-based chemotherapy, followed by four cycles of docetaxel and 1 year of trastuzumab, starting either before surgery with the first cycle of docetaxel and continuing after surgery (neoadjuvant trastuzumab arm,  $n=43$ ), or only after surgery (adjuvant trastuzumab arm,  $n=37$ ).

**Results:** *PIK3CA* mutations were found in 17 tumours (21.3%). Better disease-free survival (DFS) was observed in patients with *PIK3CA* wild-type compared with mutated tumours ( $P=0.0063$ ). By combining *PIK3CA* status and treatment arms, four separate prognostic groups with significantly different DFS ( $P=0.0013$ ) were identified.

**Conclusion:** These results confirm that the outcome of HER2-positive patients treated with trastuzumab is significantly worse in patients with *PIK3CA*-mutated compared with wild-type tumours.

The phosphatidylinositol 3-kinase (PI3K) pathway has been identified as an important player in cancer development and progression. Upon receptor tyrosine kinase activation, the PI3K kinase phosphorylates inositol lipids to phosphatidylinositol-3,4,5-trisphosphate. PI3K is a heterodimeric enzyme composed of a p110 $\alpha$  catalytic subunit encoded by the *PIK3CA* gene and a p85 regulatory subunit encoded by the *PIK3R1* gene. Phosphatidylinositol-3,4,5-trisphosphate activates the serine/threonine kinase AKT,

which in turn regulates several signalling pathways controlling cell survival, apoptosis, proliferation, motility, and adhesion (Zhao and Vogt, 2008; Baselga, 2011).

Recent reports suggest that the PI3K pathway activation could negatively influence response to trastuzumab therapy. This observation was described on both retrospective and prospective patient series (Dave *et al*, 2011; Wang *et al*, 2011; Jensen *et al*, 2012). Jensen *et al* (2012) described a statistically significant poorer

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survival in 240 HER2-positive breast cancer patients with *PIK3CA* mutations treated with trastuzumab and chemotherapy in the adjuvant setting.

*PIK3CA*, encoding one of the two PI3K subunits, is an oncogene exhibiting gain-of-function mutations in several cancers, including breast, colorectal or endometrial cancer. These mutations are present in 20–40% cases of breast cancer. *PIK3CA* is frequently mutated at hotspots in exons 9 and 20, corresponding to the helical and kinase domains, respectively (Saal *et al*, 2005; Stemke-Hale *et al*, 2008; Zhao and Vogt, 2008; Baselga, 2011). In this study, we assessed the influence of *PIK3CA* mutations on patient survival in a series of HER2-positive breast cancer patients treated with neoadjuvant chemotherapy and 1 year of trastuzumab, starting either before surgery with the first cycle of docetaxel and continuing after surgery, or only after surgery.

## MATERIALS AND METHODS

Tumour samples from 80 HER2-positive breast cancer patients were tested. All patients were participating in the phase II randomized neoadjuvant Remagus 02 trial (Pierga *et al*, 2010). The study was approved by the French Ethics Committee (03-55, RO2), and patients gave their written informed consent. All patients preoperatively received four cycles of anthracycline-based chemotherapy, followed by four cycles of docetaxel and 1 year of trastuzumab, starting either before surgery with the first cycle of docetaxel and continuing after surgery (neoadjuvant-trastuzumab arm,  $n = 43$ ), or only after surgery (adjuvant-trastuzumab arm,  $n = 37$ ). Complete follow-up data were available for the entire patient series with a median follow-up of 51 months (range: 7–76 months).

Frozen pretreatment tumour biopsies from the patients were used for total RNA extraction. *PIK3CA* mutations were detected by screening cDNA fragments obtained by RT-PCR amplification of exons 9 and 20 and their flanking exons. Details of the primers and PCR conditions are available on request. The amplified products were sequenced with the BigDye Terminator kit on an ABI Prism 3130 automatic DNA sequencer (Applied Biosystems, Courtaboeuf, France), and the sequences were compared with the corresponding cDNA reference sequence (NM\_006218).

Response to neoadjuvant therapy was determined as pathological complete response (pCR). Follow-up data for disease-free survival (DFS) and overall survival were analysed using the Kaplan-Meier method, and comparisons between groups were performed with a log-rank test.

## RESULTS

*PIK3CA* mutations were found in 17 tumours (21.3%), of which 4 were in exon 9 and 13 were in exon 20. No significant associations were found between *PIK3CA* mutations and classical clinicopathological characteristics (Supplementary Table S1). No significant difference in pCR was observed between *PIK3CA*-mutated and wild-type tumours.

Survival analysis found significantly lower DFS in *PIK3CA*-mutated cases in the overall population ( $P = 0.0063$ ; Figure 1). More detailed analysis of the four-patient subgroups based on treatment arm and *PIK3CA* status demonstrated statistically significant differences in patient outcome ( $P = 0.0013$ ; Supplementary Figure S1). The most favourable survival was observed in the subgroup of patients without *PIK3CA* mutations treated in the neoadjuvant trastuzumab arm and the poorest prognosis was observed in the subgroup of patients with *PIK3CA* mutations treated in the adjuvant trastuzumab arm. Overall

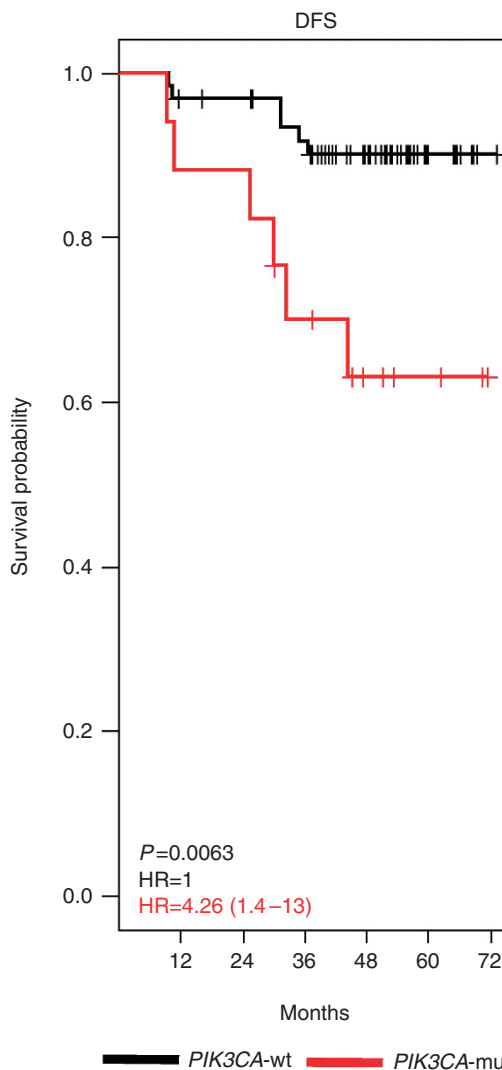


Figure 1. Disease-free survival (DFS) curves according to *PIK3CA* status in the overall population. wt = wild type.

survival curves also differed significantly in the overall population ( $P = 0.035$ ) and in the treatment-based subgroups ( $P = 0.028$ ) in favour of *PIK3CA* wild-type tumours (data not shown).

## DISCUSSION

*PIK3CA* is the most frequently mutated oncogene in human breast cancers and shows activating mutations ranging from 10% in the triple-negative subgroup to 40% in the hormonal receptor-positive/ERBB2-negative subgroups. Moreover, *PIK3CA*-mutated status confers a more favourable outcome in breast cancer patients without trastuzumab treatment (Baselga, 2011). We confirm previously published data, showing *PIK3CA* mutations in exon 9 and 20 hotspots in about 20% of HER2-positive breast cancers and occurring more frequently in exon 20 (Baselga, 2011; Dave *et al*, 2011; Jensen *et al*, 2012). In the present study focusing on 1 year of trastuzumab treatment, patients with *PIK3CA*-mutated tumours had a poorer outcome than *PIK3CA* wild-type cases (Figure 1). A favourable survival benefit was observed when neoadjuvant trastuzumab was added early to neoadjuvant chemotherapy, particularly in patients with *PIK3CA* wild-type tumours (Supplementary Figure S1).

These data therefore support the negative influence of PI3K pathway activation on response to trastuzumab therapy described by Jensen *et al* (2012). Moreover, based on a larger series, we confirm the data reported by Dave *et al* (2011), who studied the effects of *PIK3CA* mutations on response to neoadjuvant trastuzumab therapy in a small series of 32 HER2-positive breast cancer patients. It is noteworthy that these authors similarly did not find any difference in pCR associated with *PIK3CA* mutations. Importantly, the results described here are derived from a prospective clinical trial of neoadjuvant patients with pretreatment tumour samples available for assessment and with well-documented follow-up. Thus, the mutational status assigned to each patient showed the therapy-naive tumour condition before initiation of study treatment. This is an important point, especially in the light of a report by Dupont Jensen *et al* (2011), showing discordances between *PIK3CA* mutations in primary breast tumours and their metastases, which might influence the results of studies based on retrospective sample collection and advanced treatment lines.

Furthermore, the negative effect of *PIK3CA* mutations on response to trastuzumab therapy is also supported by similar observations in breast cancer cell lines (Berns *et al*, 2007; Dave *et al*, 2011; Jensen *et al*, 2012). This extends and underlines the knowledge of the effect of *PIK3CA* mutations and PI3K pathway activation on HER2-inhibitor treatment response observed on patient breast tumour samples. In the light of published data, PI3K pathway activation also appears to predict treatment response to the HER2-targeting tyrosine kinase inhibitor lapatinib (Eichhorn *et al*, 2008).

Altogether, these data suggest that only *PIK3CA* wild-type cancers clearly benefit from neoadjuvant trastuzumab therapy added to chemotherapy. On the other hand, the subgroup of patients bearing *PIK3CA* mutations could further benefit from treatment targeting PI3K pathway signalling (PI3K or its downstream major effectors; Kataoka *et al*, 2010; Tanaka *et al*, 2011; Jensen *et al*, 2012). Such treatment may be able to overcome the activation effect of *PIK3CA* mutations and block the PI3K pathway signalling. Our results support the importance of *PIK3CA* mutational status assessment in the management of future gene-based therapies (HER2, mTOR or PI3K inhibitors used alone or in combination) for HER2-positive breast cancer.

In conclusion, these results confirm that *PIK3CA* mutations are a pejorative factor in HER2-positive breast cancer patients receiving trastuzumab. *PIK3CA* mutations should be assessed in clinical trials testing anti-HER2 therapies and, in the future, in clinical practice.

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## REFERENCES

- Baselga J (2011) Targeting the phosphoinositide-3 (PI3) kinase pathway in breast cancer. *Oncologist* **16**(Suppl 1): 12–19.
- Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez-Angulo AM, Stemke-Hale K, Hauptmann M, Beijersbergen RL, Mills GB, van de Vijver MJ, Bernards R (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* **12**: 395–402.
- Dave B, Migliaccio I, Gutierrez MC, Wu MF, Chamness GC, Wong H, Narasanna A, Chakrabarty A, Hilsenbeck SG, Huang J, Rimawi M, Schiff R, Arteaga C, Osborne CK, Chang JC (2011) Loss of phosphatase and tensin homolog or phosphoinositide-3 kinase activation and response to trastuzumab or lapatinib in human epidermal growth factor receptor 2-overexpressing locally advanced breast cancers. *J Clin Oncol* **29**: 166–173.
- Dupont Jensen J, Laenkholm AV, Knoop A, Ewertz M, Bandaru R, Liu W, Hackl W, Barrett JC, Gardner H (2011) PIK3CA mutations may be discordant between primary and corresponding metastatic disease in breast cancer. *Clin Cancer Res* **17**: 667–677.
- Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, Beijersbergen RL, Valero V, Seoane J, Bernards R, Baselga J (2008) Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. *Cancer Res* **68**: 9221–9230.
- Jensen JD, Knoop A, Laenkholm AV, Grauslund M, Jensen MB, Santoni-Rugiu E, Andersson M, Ewertz M (2012) PIK3CA mutations, PTEN, and pHER2 expression and impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab. *Ann Oncol* **23**: 2034–2042.
- Kataoka Y, Mukohara T, Shimada H, Saijo N, Hirai M, Minami H (2010) Association between gain-of-function mutations in PIK3CA and resistance to HER2-targeted agents in HER2-amplified breast cancer cell lines. *Ann Oncol* **21**: 255–262.
- Pierga JY, Delaloge S, Espié M, Brain E, Sigal-Zafrani B, Mathieu MC, Bertheau P, Guinebrière JM, Spielmann M, Savignoni A, Marty M (2010) A multicenter randomized phase II study of sequential epirubicin/cyclophosphamide followed by docetaxel with or without celecoxib or trastuzumab according to HER2 status, as primary chemotherapy for localized invasive breast cancer patients. *Breast Cancer Res Treat* **122**: 429–437.
- Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmström PO, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R (2005) PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res* **65**: 2554–2559.
- Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, Hung MC, van de Vijver MJ, Valero V, Gray JW, Bernards R, Mills GB, Hennessy BT (2008) An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* **68**: 6084–6091.
- Tanaka H, Yoshida M, Tanimura H, Fujii T, Sakata K, Tachibana Y, Ohwada J, Ebiike H, Kuramoto S, Morita K, Yoshimura Y, Yamazaki T, Ishii N, Kondoh O, Aoki Y (2011) The selective class I PI3K inhibitor CH5132799 targets human cancers harboring oncogenic PIK3CA mutations. *Clin Cancer Res* **17**: 3272–3281.
- Wang L, Zhang Q, Zhang J, Sun S, Guo H, Jia Z, Wang B, Shao Z, Wang Z, Hu X (2011) PI3K pathway activation results in low efficacy of both trastuzumab and lapatinib. *BMC Cancer* **11**: 248.
- Zhao L, Vogt PK (2008) Class I PI3K in oncogenic cellular transformation. *Oncogene* **27**: 5486–5496.

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