Editorial Commentary | Biomarkers Sciences

The quest for the optimal biomarker: is extending the spectrum of targeted *PIK3CA* mutations in breast cancer carcinoma worthwhile?

Stanislas Quesada, William Jacot

Institut régional du Cancer de Montpellier (ICM), INSERM U1194, Montpellier University, Montpellier, France

Correspondence to: William Jacot, MD, PhD. Institut régional du Cancer de Montpellier, Parc Euromédecine, 208 Avenue des Apothicaires, 34090 Montpellier, France. Email: william.jacot@icm.unicancer.fr.

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In the context of hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-negative ($HR^+/HER2^-$) advanced breast cancers (ABCs), dysregulation of the phosphatidylinositol 3-kinase (PI3K) pathway has been has been implicated in initial or acquired resistance to endocrine therapy (ET) with or without cyclin-dependent kinase 4 and 6 inhibitor (CDK4/6i) and is associated with worse overall survival (OS) (1,2). Indeed, PI3K upregulation have been reported in up to 40% of $HR^+/HER2^-$ ABCs, mainly by sequencing a few hotspot mutations in the PI3K catalytic subunit alpha (*PIK3CA*) gene (3,4). *PIK3CA* encodes p110α, the catalytic subunit of PI3Kα that associates with the p85 regulatory subunit. This regulatory subunit stabilizes and inhibits the kinase activity of p110α. Briefly, following fixation of growth ligand on tyrosine kinase receptor (RTK), PI3Kα is recruited to plasma membrane and is activated, leading to phosphorylation of phosphatidylinositol 4,5 biphosphate (PIP2) towards phosphatidylinositol 3,4,5 triphosphate (PIP3) (5). This phosphorylation is the starting point of a phosphorylation-based signaling cascade, which, in fine, exerts pro tumorigenic functions such as metabolic reprogramming, aberrant cellular growth and survival.

As such, PI3Kα inhibition has been an intense field of therapeutic development this last decade, as a way to circumvent ET-resistance (6). Following this perspective, alpelisib (a PI3Kα-specific inhibitor) has been developed, with positive early phase studies (7). Subsequently, the SOLAR-1 trial (NCT02437318) evaluated the adjunction of alpelisib (*vs.* placebo) to fulvestrant for the treatment of $HR^+/HER2^-$ ABCs following progression under ET (8). Patients were enrolled in two cohorts depending on their tumor-based *PIK3CA* mutation status. Noteworthy, this status was evaluated through hotspot-directed molecular assay, focused on 11-point mutations affecting exons 7, 9, and 20 (SOLARm*): C420R, E542K, E545K/G/A/D, Q546E/R and H1047R/L/Y. These hotspot mutations, found in 80% of *PIK3CA*-mutated ABCs, were selected based on their oncogenic potential through enhanced enzymatic activation (2,5). The primary endpoint was investigator-assessed progression-free survival (PFS) in the *PIK3CA* mutant cohort. The *PIK3CA*-mutated cohort reported a median PFS of 11.0 months [95% confidence interval (95% CI): 7.5–14.5] in the fulvestrant plus alpelisib (Ful/Alp) arm compared with 5.7 months (95% CI: 3.7–7.4) in the fulvestrant plus placebo arm [hazard ratio (HR), 0.65; 95% CI: 0.50–0.85; P<0.001]. Conversely, the second "non-mutated" cohort did not exhibit advantage of adding alpelisib (HR, 0.85; 95% CI: 0.58–1.25). Following publication of this pivotal trial, the Food and Drug Administration (FDA) approved on May 24, 2019 the Ful/Alp combination for patients with *PIK3CA*-mutated (SOLARm*) $HR^+/HER2^-$ ABCs who had previously received ET-based regimen, in association with

the Therascreen® PIK3CA RGQ PCR Kit (QIAGEN® Manchester Ltd., Manchester, UK) companion diagnostic assay for SOLARm* detection (9). Subsequently, the European Medicines Agency (EMA) approved Ful/Alp on May 28, 2020 for the treatment of postmenopausal women, and men, with $HR^+/HER2^-$ locally advanced or metastatic breast cancer with a *PIK3CA* mutation after disease progression following ET as monotherapy (10).

Apart from enlarging the therapeutic panel in the $HR^+/HER2^-$ ABC population, this pivotal trial raised several questions, including the optimal way to select the target population. Indeed, relevance of Ful/Alp for ABCs with non-SOLARm* *PIK3CA* mutations was not directly evaluated as, in SOLAR-1, the "non-mutated" cohort consisted actually of patients with either wild type or *PIK3CA*-mutated tumors outside of the SOLARm* list of mutations, as only 11 mutations were considered in the trial. This was one of the main question of a recent study published in *Clinical Cancer Research* by Rugo and colleagues, which reported an unprecedented cohort of 33,977 retrospectively evaluated $HR^+/HER2^-$ ABC patients, for whom an analysis of the whole coding-sequence of *PIK3CA* gene through next-generation sequencing (NGS) was available (11). NGS was performed using either the FoundationOne (F1) or FoundationOne CDx (F1-CDx) assays (Foundation Medicine® Inc., Cambridge, MA, USA), allowing analysis of panels of 287 and 324 genes, respectively. Furthermore, circulating cell-free DNA (cfDNA) analyses were performed on 1,587 patients using the FoundationOneLiquid CDx assay (F1-LCDx). From the initial cohort of 33,977 patients, predicted pathogenic mutations of *PIK3CA* were detected in 11,767 cases (35%). Noteworthy, 2,300 (7%) of these cases displayed non-SOLARm* alterations and 1,216 (4%) had combinatorial (e.g., SOLARm* + non-SOLARm*) *PIK3CA* mutations. In other words, one fifth of patients with *PIK3CA* mutations detected by NGS on tissues would have been missed by gold standard testing dedicated to the sole SOLARm*. A few SOLARm* represented the most prevalent mutations detected (% of *PIK3CA* cases on tissue analyses): H1047R (38%), E545K (21%) and E542K (13%). Conversely, some of other SOLARm* were vanishingly rare: E545D (0.12%), Q546E (0.20%) and H1047Y (0.27%). Strikingly, several non-SOLARm* exhibited high prevalence among *PIK3CA*-mutated cases, such as N345K (5.7%) and E726K (3.2%). Surprisingly, indels affecting the p85 binding domain represented 3.1% of mutated cases, a portion of the gene that was not considered by the SOLARm* panel. When considering the oncogenic potency of non-SOLARm* (i.e., driver *vs.* secondary mutations), it appeared that subclonal mutations (algorithmically predicted using the somatic-germline-zygosity method) tended to be commutated with SOLARm*. This latter point may be suggestive of mutations appearing later during tumor evolution, enhancing SOLARm*-mediated PIK3CA activation. Conversely, mutations that were highly represented in non-SOLARm* subset (e.g., N345K and indels in the p85 binding domain) tended to be mutually exclusive with SOLARm*, suggesting that they may act as true oncogenic drivers. Interestingly, mutational landscape of tumors with SOLARm* or with non-SOLARm* exhibited striking similarity, suggesting analogous carcinogenetic pathways.

Regarding analyses on liquid biopsies, cfDNA was detected in 94% of cases and the rate of detection of *PIK3CA* mutations was 42%, of whom 19.1% were non-SOLARm*. Furthermore, a concordance analysis was performed on a subset of 206 patients with matched tissue- *vs.* cfDNA-based techniques, exhibiting a global 77% positive percent agreement (PPA). Of note, *PIK3CA* mutations detected in tissue only tended to have lower variant allele frequencies in the tissue biopsy. Furthermore, *PIK3CA* mutation detection with cfDNA increased to 95% PPA when tumor fraction within cfDNA was ≥2% (78% of the paired cohort) and reached 100% when tumor fraction was ≥10% (78% and 37% of the paired cohort, respectively), raising the necessity of sufficient tumor fraction for accurate detection of mutations. Subsequently, based on the Flatiron Health-Foundation Medicine clinico-genomic database (CGDB), a retrospective analysis was performed on 10,750 of these patients. Based on CGDB, a subset of *PI3KCA*-mutated (including both SOLARm* and non-SOLARm*) $HR^+/HER2^-$ ABC patients were analyzed: 182 patients treated with Ful/Alp and 119 with fulvestrant alone and a median PFS of 5.9 (95% CI: 5.1–7.4) and 3.1 (95% CI: 2.7–3.7) months was reported (HR, 0.61; 95% CI: 0.50–0.75; P<0.0001; Ful/Alp and fulvestrant, respectively). Subsequently, authors assessed Ful/Alp efficacy according to the type of *PIK3CA* mutations. In patients with SOLARm* tumors (Ful/Alp n=150; fulvestrant n=92), combination therapy led to a median PFS of 6.8 (95% CI: 5.9–8.6) *vs.* 3.2 (95% CI: 2.7–3.7) months for fulvestrant alone (HR, 0.61; 95% CI: 0.50–0.75; P<0.0001). When restricting to non-SOLARm* tumors (Ful/Alp n=32; fulvestrant n=27), median PFS were as follows: 4.0 (95% CI: 2.8–10.1) and 2.5 (95% CI: 2.2–3.7) months (HR, 0.54; 95% CI: 0.34–0.84; P<0.0054; Ful/Alp and fulvestrant, respectively). Of

note, regarding tumors combining SOLARm* and non-SOLARm* *PI3KCA* co-mutations (n=44) a non-significant HR of 0.68 (0.40–1.16) was reported.

This study raises several interesting insights. First, non-invasive *PIK3CA* evaluation appears feasible and may be of interest, notably in the context of ABC patients without easy-to-analyze tissue (e.g., ABCs with bone metastases only), however with the necessity of sufficient tumor fraction within cfDNA. Indeed, sufficient tumor fraction (and consequently its metric) appears of prime importance prior to draw a definite conclusion regarding a cfDNA-based result. Secondly, a positive impact of adding alpelisib to fulvestrant is seen in real-life practice, notably in the current ET + CDK4/6i frontline combination population. Indeed, in the pivotal SOLAR-1 trial, only a minority of patients previously received ET + CDK4/6i before randomization (5.3%). This reinforces the previously reported data from the non-comparative, phase 2, BYLieve trial (12). Ongoing EPIK-B5 (NCT05038735) phase 3 trial, comparing fulvestrant + placebo *vs.* fulvestrant + alpelisib following progression under ET + CDK4/6i frontline combination aims at validating this strategy. Thirdly, apart from initial FDA approval based on a restricted panel of 11 hotspot mutations detected with dedicated companion diagnostic assays (CDx), alpelisib seems to still exert antitumor effect on ABCs bearing other *PIK3CA* mutations. This point is even more important in the context of current management of ABCs, where NGS with large panels is a common strategy, leaving physicians with unanswered questions when a potently pathogenic non-SOLARm* is reported. As such, alpelisib prescription for non-SOLARm* *PIK3CA*-mutated $HR^+/HER2^-$ ABCs could be considered, following validation in molecular tumor board, and would increase the target population by approximately 20%. The impact of non-SOLARm* is of prime importance in currently recruiting clinical trials, where alpelisib is currently positioned in frontline maintenance, notably in the EPIK-B2 (NCT04208178) and SAFIR 03 (NCT05625087) trials. However, prior to its implementation in clinical routine, supplementary studies appear necessary, both at the molecular and clinical scales. Indeed, while extensive characterization of functional impact of E545K and H1047R have been reported so far, such an effort for rarer mutations described in the present study is required. Interestingly, regarding non-SOLARm* reported mutations, apart from alterations affecting well-characterized exons 7, 9 and 20, a distinct cluster of mutations affecting p85 binding-domain and ABD/RAS hinge region (corresponding to exons 1 and 2) have been reported in this study. Interestingly, this kind of mutations have been previously reported in endometrial malignancies (13). At the molecular scale, it is thought that mutations affecting this region of p110 may inhibit its interaction with p85 and/or facilitate its kinase activity (14).

Finally, a specific insight regarding tumors with *PI3KCA* co-mutations should be considered. Indeed, previous studies exhibited that co-mutations in ABC's could represent 12–15% of *PIK3CA*-mutated cases and that these co-mutations lead to enhanced oncogenicity (15). Nevertheless, this specific subpopulation of co-mutations represented only 4% (n=44) of the cohort presented by Rugo and colleagues (11). Furthermore, with an HR of 0.68 (0.40–1.16), the present study did not report sensitivity to Ful/Alp in the context of *PI3KCA* co-mutations. This represents one of the unassessed points of the present study. Indeed, a recent ancillary study from the SANDPIPER trial (which evaluated the adjunction of taselisib—another specific inhibitor of the PI3K pathway—to fulvestrant for the treatment of $HR^+/HER2^-$ ABCs following progression under ET) reported that patients with clonal *PIK3CA* co-mutations detected on cfDNA exhibited enhanced PFS and that they could be of interest as predictive biomarkers (16).

Collectively, data from Rugo *et al.* (11) raise the importance of comprehensive genomic profiling of patients with HR^+ ABC's rather than simply "hotspot-focused" CDx; and pledge for considering cfDNA as a suitable alternative in this context.

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