Reviewer Comments

Reviewer A

Comment 1: The commentary is well written, distills the main messages of the Rugo et al. study and provides context for the findings.

Reply 1: The authors sincerely thank reviewer A for relevant comments. All of these have been integrated in the final version of the manuscript.

Comment 2: Line 17: Dysregulation of the phosphatidylinositol 3-kinase (PI3K) pathway has been shown to participate to initial or acquired resistance to endocrine therapy (ET) +/- cyclin-dependent kinase 4 and 6 inhibitor (CDK4/6i) and worse overall survival (OS).

Consider rephrasing to: dysregulation of the phosphatidylinositol 3-kinase (PI3K) pathway **has been implicated in initial or acquired resistance** to endocrine therapy (ET) +/- cyclin-dependent kinase 4 and 6 inhibitor (CDK4/6i) and is associated with worse overall survival (OS).

Reply 2: Sentences reformulated according to the proposal

Comment 3: Line 19: Indeed, PI3K upregulation have been reported in up to 40% of HR+/HER2-ABCs, mainly by sequencing for few hotspot mutations in the PI3K catalytic subunit alpha (PIK3CA) gene (3,4).

Indeed, PI3K upregulation has 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).

Reply 3: Sentences reformulated according to the proposal

Comment 4: Line 35: These hotspot mutations, corresponding to roughly 80% of all detected PIK3CA mutation, were selected based on their oncogenic potential through enhanced enzymatic activation (2,5).

These hotspot mutations**, found in 80% of patients with detected PIK3CA mutations,** were selected based on their oncogenic potential through enhanced enzymatic activation (2,5).

[The Rugo et al. study and PMID: 32404150 both find 80% of *patients* have one of these hotspot mutations, but these 11 hotspots represent ~70% of total PIK3CA mutations.]

Reply 4: Thank you for this interesting suggestion, we reformulated accordingly

Comment 5: Line 38: 95% confidence interval abbreviation 95CI needs to be defined at the first use.

Reply 5: We corrected this mistake

Comment 6: Line 63: In other words, one-fifth of all detected PIK3CA mutations detected by NGS on tissues would have been missed by gold standard testing dedicated to the sole SOLARm*.

In other words, one-fifth **of _patients_ with PIK3CA mutations detected by NGS on tissue** would have been missed by gold standard testing dedicated to the sole SOLARm*.

[Same reason as the comment on Line 35: (2300/11767 of patients)]

Reply 6: We corrected this mistake

Comment 7: Line 82: Following the same trend, PIK3CA mutation detection with cfDNA increased to 95% PPA when **cfDNA** fraction was ≥2% (78% of the paired cohort) and reached 100% when **fraction** was ≥10% (78% and 37% of the paired cohort, respectively).

[the fraction is the tumor fraction within cfDNA. So it should be called tumor fraction or circulating tumor DNA (ctDNA) fraction]

Reply 7: Thanks for the relevant input, we corrected to "tumor fraction within cfDNA"

Comment 8: Line 84: Subsequently, based on the Flatiron Health-Foundation Medicine **clinico genomic database** (**CGDB**),

Reply 8: We actually used the Flatiron Health- Foundation Medicine clinico genomic database acronym. We nevertheless modified it according to your proposal

Comment 9: Line 99: However with the necessity of sufficient **cfDNA** fraction.

[Tumor fraction or ctDNA fraction, same reason as line 82 comment]

[Suggest adding a brief sentence alluding to the importance of assays providing this tumor fraction metric to help clinicians determine whether a negative result is trustworthy or needs to be verified by a different test.]

Reply 9: Thanks for the relevant input, we corrected to "tumor fraction within cfDNA"

Reviewer B
Comment 1: This manuscript provides commentary on an important translational topic and an important recent publication by HS Rugo et al. ("Biology and targetability of the extended spectrum of PIK3CA mutations (PIK3CAm) detected in breast carcinoma". Clinical Cancer Research. 2023 Mar 14;29(6):1056-1067.). Previous clinical studies in breast cancer of the PI3K inhibitor alpelisib (BYL719) have focused on stratifying patients according to hotspot PIK3CA mutations occurring at 5 amino acids. However, breast cancer tumors are known to contain mutations at multiple other

non-hotspot sites, as detected by Rugo et al., and there is reasonable accumulating evidence that alpelisib should also be used for patients whose tumors harbor these non-hotspot mutations. This would expand the pool of patients who may benefit from alpelisib therapy. The current commentary addresses this possibility.

Reply 1: The authors sincerely thank reviewer A for relevant comments. All of these have been integrated into the final version of the manuscript.

Comment 2: Lines 19-21 ["Indeed, PI3K upregulation have been reported in up to 40% of HR+/HER2- ABCs, mainly by sequencing for few hotspot mutations in the PI3K catalytic subunit alpha (PIK3CA) gene (3,4)."] confusingly and errantly connects "upregulation" with hotspot mutation. Upregulation refers to expression, whereas mutation would refer to functional activity.

Reply 2: The authors sincerely thank reviewer A for relevant comments. All of these have been integrated into the final version of the manuscript.

Comment 3: In lines 35-36 ["These hotspot mutations, corresponding to roughly 80% of all detected PIK3CA mutation…"] it is unclear what is meant by "of all detected PIK3CA mutation". All detected PIK3CA mutations in all cancers? In breast cancer alone? In The Cancer Genome Atlas studies? In studies by some other group?
Reply 3: We changed the sentence to : "These hotspot mutations, found in 80% of *PIK3CA*-mutated ABCs,, were selected based on their oncogenic potential through enhanced enzymatic activation"

Comment 4: In lines 60-61 ["In this population, predicted pathogenic mutations of PIK3CA were detected in 11 767 cases (35%)."], one can only guess that this 35% refers to the 33,977 patients mentioned earlier, and not the 1,587 patient subpopulation mentioned in the immediately preceding sentence.

Reply 4: We changed the sentence to: 'From the initial cohort of 33 977 patientsIn this population, predicted pathogenic mutations of PIK3CA were detected in 11 767 cases (35%)"

Comment 5: Lines 70-72 ["When considering the oncogenic potency of non-SOLARm* (i.e. driver vs passenger mutations), it appeared that subclonal mutations tended to be comutated with SOLARm*, suggesting that they appeared later during cancer progression."] is not understandable. Also, what is meant by "subclonal mutations"?
Reply 5: We changed it to : 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 comutated 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.

Comment 6: Line 81, what does "VAF" stand for?

Reply 6: Thank you for highlighting this point. This abbreviation is intended for Variant allele frequency (developed version has been added).

Comment 7: Line 82, what does "PPA" stand for?

Reply 7: This abbreviation is used for positive percent agreement, which is explained before.

Comment 8: In lines 82-84 ["Following the same trend, PIK3CA mutation detection with cfDNA increased to 95% PPA when cfDNA fraction was ≥2% (78% of the paired cohort) and reached 100% when fraction was ≥10% (78% and 37% of the paired cohort, respectively)."] it is unclear what is being communicated.

Reply 8: We changed it to: 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

Comment 9: Line 120, the second "p110" mentioned should be changed to "p85"

Reply 9: Thanks for highlighting this misprint, we corrected it accordingly.

Comment 10: Line 134, what does "CDx" stand for?

Reply 10: This abbreviation is used for "companion diagnostic assays". We added its definition.