Review of: "p16INK4A-deficiency predicts response to combined HER2 and CDK4/6 inhibition in HER2+ breast cancer brain metastases"

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Commentary:

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This study puts forth the hypothesis that deficiency in the tumor suppressor $p16^{INK4A}$ predicts response to combination therapy of tucatinib, a HER2+ inhibitor with CNS penetration, and abemaciclib, a CDK4/6 inhibitor also with CNS penetration, in HER2+ breast cancer brain metastases (BCBMs) and tests this hypothesis with a four-part experiment. All four parts examine response to monotherapy of both tucatinib and abemaciclib as well as combination therapy but in different models of orthotopic tumors. The first part found that monotherapy had little effect in DFBM-355 (deficient in $p16^{INK4A}$ and null in the corresponding gene CDKN2A), whereas combination therapy resulted in marked tumor regression and increased survival in the mice. The second part found that the model DFBM-727, *proficient* in both $p16^{INK4A}$ and CDKN2A copy number variants, responded to tucatinib monotherapy without further response upon addition of abemaciclib. In the third part, the authors tested two models, DFBM-Ni8 and DFBM-Nil7, both deficient in $p16^{INK4A}$ but with one and two CNVs of CDKN2A, respectively. In both cases, combination therapy resulted in tumor regression and stable disease in the mice. Finally, the fourth part of the experiment restored $p16^{INK4A}$ expression in the otherwise deficient DFBM-Nil7 model in an acute manner so as to avoid any confounding variables from the long-term expression of the protein. Upon this restoration, the tumors responded to tucatinib monotherapy alone.

The first merit of this study is its comprehensive, four-part design. In the first model, the suggestion is that because tucatinib alone was ineffective, the absence of CDKN2A and the corresponding deficiency in $p16^{INK4A}$ confer resistance to tucatinib. The model's response to combination therapy with the CDK4/6 inhibitor abemaciclib further suggests that the mechanism of resistance is upregulation of CDKs. The second part tests the inverse of the first, with a model that is proficient in both the gene and protein, and correspondingly elicited the opposite response, i.e. improvement upon rather than resistance to tucatinib monotherapy, thereby solidifying the findings in the first part. The third section importantly makes a distinction between the gene and the protein, using models that have CNVs of CDKN2A but are nonetheless deficient in $p16^{INK4A}$. This distinction is important because the authors found that the number of CNVs does not linearly predict the extent of protein expression, and in fact, a majority of the HER2+ xenografts showed significant variation in

CDKN2A mRNA expression while remaining deficient in $p16^{INK4A}$. The fact that these two models demonstrated the same response as the model in the first part of the experiment suggests that it is specifically protein deficiency rather than quantity of CNVs or mRNA expression that predicts a positive response to combination therapy. Finally, the findings in the fourth part support those in the third by demonstrating that only upon acute expression of $p16^{INK4A}$ did the model responded to tucatinib monotherapy. Taken together, these four parts allow the authors to show a clear association between $p16^{INK4A}$ deficiency and BCBM response to tucatinib-abemaciclib combination therapy without going so far as causality.

The second merit of this study is its novel contribution to the literature, combining various topics that exist in isolation in the literature into a cohesive hypothesis. For example, there is work consistent with this study on how $p16^{INK4A}$ deficiency may be implicated in breast cancer proliferation and migration [Yang]. There is also research on the efficacy of tucatinib monotherapy [Ulrich] and abemaciclib monotherapy, both in HER2- [NCT03155997] and to a lesser degree in HER2+ [Patnaik] breast cancer, as well as abemaciclib's ability to cross the BBB to reach brain metastases [Raub]. Some literature even makes the step to bring the topics of p16 deficiency and tucatinib-abemaciclib combination therapy together, though in the context of gastric cancer [Bae] and relapsed mesothelioma [Fennell] rather than BCBMs. To the extent that these findings extrapolate to BCBMs, however, they too are consistent with this study. None, however, marry all of these moving parts into the cohesive hypothesis that 1) deficiency in $p16^{INK4A}$ 2) predicts positive response 3) to tucatinib-abemaciclib combination therapy 4) in HER2+ BCBMs.

As a final merit, this study may also prove to be the basis for a novel clinical trial and perhaps ultimately a novel therapy. Currently, the FDA has approved tucatinib (Tukysa®) with trastuzumab or capecitabine in HER2+ breast cancer [FDA reference ID: 4593756] and abemaciclib (Verzenio®) with tamoxifen or an aromatase inhibitor such as fulvestrant in HR+ HER2- breast cancer [FDA reference ID: 4226487]. A now completed clinical trial also tested the efficacy of abemaciclib as a single agent or with endocrine therapy in both HR+ HER2+ and HR+ HER2- breast cancer [NCT02308020], while the currently active monarcHER trial tests trastuzumab alone, with abemaciclib, and with both abemaciclib and fulvestrant in HR+ HER2+ breast cancer [NCT02675231]. Correspondingly, the American Society of Clinical Oncology's 2022 guidelines for HER2+ breast cancer recommend tucatinib with trastuzumab or capecitabine as third-line treatment and abemaciclib with trastuzumab or fulvestrant, also as a third-line treatment [Giordano]. However, the combination of tucatinib and abemacicilb has, to date, never undergone clinical trial. Because the authors of this study found that not only was there an association between $p16^{INK4A}$ deficiency and response to combination therapy but also that, in fact, a *majority* of the xenografts were deficiency in $p16^{INK4A}$, thereby imparting a wider applicability to their study, they hope to have established the rationale for a biomarker-driven clinical trial of a HER2+ targeted agent with a CDK4/6 inhibitor.

Despite these merits, the authors may wish to more clearly articulate their rationale for selecting the combination of tucatinib and abemacicilb specifically. The selection of tucatinib is likely due to its high selectivity for HER2, as opposed to EGFR and HER4, which the other small TKIs also target [Schlam]. In the case of abemacicilb, however, there is evidence that palbociclib or ribociclib may also constitute promising treatments. For example, palbociclib showed promise against HER2+ breast cancer in combination with trastuzumab and tamoxifen during its preclinical development [Finn], while another study found that it worked synergistically with lapatinib, in the same drug class as tucatinib, also in HER2+ breast cancer [Nikolai]. An avenue that the authors and other researchers may consider in the future is comparing combination therapy of tucatinib with each of the three CDK4/6 inhibitors.

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