Evaluation of *nab*-sirolimus in combination with fulvestrant or PI3K pathway inhibitors to overcome resistance in breast cancer cell lines

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Objective

- **To determine if *nab*-sirolimus in combination with the estrogen receptor antagonist fulvestrant, or a PI3Ki, impacts cytotoxicity of anticancer agents in *PI3K*-mutated cancer cell lines**

KEY FINDINGS

- **The mTORi *nab*-sirolimus enhanced the cytotoxic effects of fulvestrant in HR+ BrCa cells and PI3Ki in both HR+ and HR– BrCa cells (Figures 2 and 3)**
- **Western blot analysis showed increased phosphorylation of phospho-4EBP1 in response to fulvestrant or PI3Ki treatment, which was reversed with the addition of *nab*-sirolimus (Figures 4 and 5)**
- **Conversely, *nab*-sirolimus caused phospho-AKT activation through a negative feedback loop, which was reversed by the combination with fulvestrant or PI3Ki (Figures 4 and 5)**
- **Addition of *nab*-sirolimus to endocrine therapy or PI3K/AKT/mTOR pathway inhibitor may mutually overcome mechanisms of resistance induced by single-agent treatments**
- **These data support a vertical PI3K pathway inhibition strategy using *nab*-sirolimus and PI3Ki in *PIK3CA*-mutated BrCa, regardless of hormone receptor status**
- **These data further support the combination of *nab*-sirolimus with endocrine therapy for hormone-driven cancers, which is currently being investigated in patients with advanced or recurrent endometrioid endometrial cancer in a phase 2 study (NCT05997017)**

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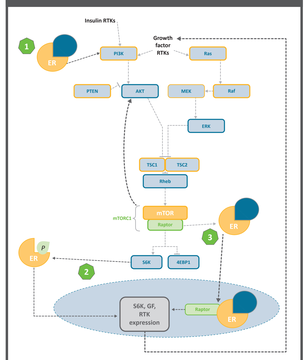
BACKGROUND

- The PI3K/AKT/mTOR pathway can activate estrogen receptor (ER) transcriptional activity even in the absence of estrogen signaling,[1] and activated ERs can directly activate the PI3K/AKT/mTOR pathway[2] (**Figure 1**)
- Crosstalk between the PI3K/AKT/mTOR pathway and ER signaling has been associated with potential mechanisms for resistance to endocrine therapy[1,3]
- Activating mutations in *PIK3CA* are found in ~40% of hormone receptor-positive (HR+) breast cancers (BrCa)[1]
- Fulvestrant, the selective ER degrader[1] and PI3K inhibitors, such as alpelisib, have demonstrated efficacy in patients with HR+ *PI3K*-mutated BrCa[3]; however, resistance still occurs[4]

Figure 1. Crosstalk between ER signaling and the PI3K/AKT/mTOR pathway



(1) Activated ERs directly bind PI3K leading to pathway activation.
(2) S6K, a downstream signaling protein can phosphorylate and activate ERs, leading to expression of several PI3K/AKT/mTOR pathway regulators, which can result in upstream activation of the pathway.
(3) Estrogen bound ERs recruit Raptor to the nucleus and promote expression of growth factors, which also activates the pathway.

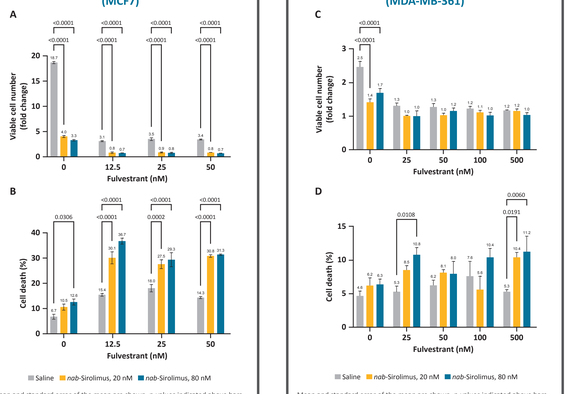
- Addition of an mTOR inhibitor (mTORi) to endocrine therapy, and combination therapy strategies to inhibit multiple PI3K/AKT/mTOR pathway elements, may overcome resistance mechanisms
- *nab*-Sirolimus is a nanoparticle, albumin-bound, IV-administered mTORi approved in the United States for the treatment of adults with advanced malignant perivascular epithelioid cell tumors (PEComa)[5]
- In prior nonclinical studies, *nab*-sirolimus demonstrated improved tumor accumulation, mTOR inhibition, and antitumor effects, compared with oral mTORis[6]
- We analyzed the effects of *nab*-sirolimus in combination with a PI3Ki or ER antagonist, fulvestrant, in *PI3K*-mutated BrCa cells

METHODS

- HR+, human epidermal growth factor receptor 2 amplified (HER2-AMP) or HER2-negative (HER2–), *PI3K*-mutated BrCa cell lines (MDA-MB-361 [HER2-AMP]; MCF7 [HER2–]) were incubated for 5 days (**Figure 2**) with clinically relevant doses of:
  - *nab*-Sirolimus
  - Fulvestrant
- HR+, HER2-AMP, *PI3K*-mutated BrCa cell line (MDA-MB-361) or HR-negative (HR–), HER2–, *PI3K*-mutated BrCa cell line (MDA-MB-453) were incubated for 5 days (**Figure 3**) with clinically relevant doses of:
  - *nab*-Sirolimus
  - PI3K pathway inhibitors gedatolisib (dual PI3K and mTOR inhibitor)[7] or alpelisib (PI3Kα-specific inhibitor)[8]
- The antiproliferative and cytotoxic effects of single-agent and combination treatment were assessed using an automated trypan blue exclusion assay
- Statistical significance of changes in cell viability and cell death were analyzed by two-way analysis of variance
- Cell signaling was analyzed by Western blot (**Figures 4** and **5**)

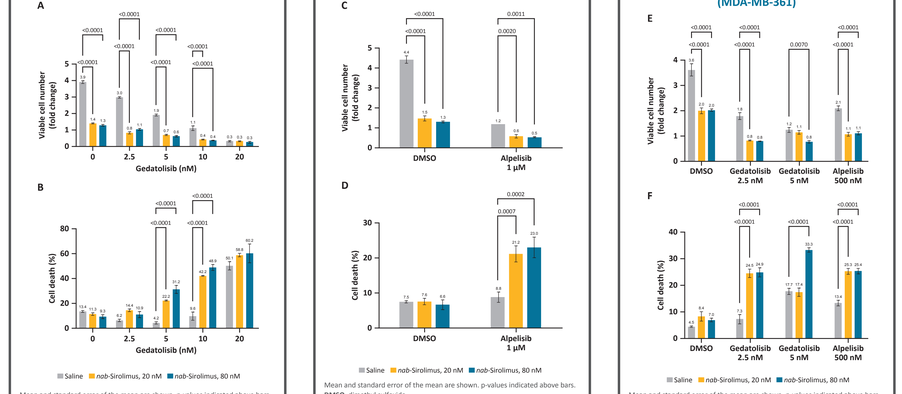
RESULTS

Figure 2. Effects of *nab*-sirolimus in combination with fulvestrant on cell proliferation (A, C) and cytotoxicity (B, D) in HR+ BrCa cell lines



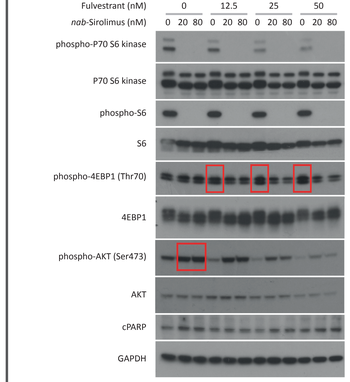
Mean and standard error of the mean are shown. p-values indicated above bars.

Figure 3. Effects of *nab*-sirolimus in combination with a PI3Ki on cell proliferation (A, C, E) and cytotoxicity (B, D, F) in HR+ and HR– BrCa cell lines



Mean and standard error of the mean are shown. p-values indicated above bars.
DMSO, dimethyl sulfoxide.

Figure 4. Western blot analysis of the effects of *nab*-sirolimus in combination with fulvestrant on key signaling molecules in HR+, HER2–, *PI3K*-mutated BrCa cells (MCF7)



cPARP, cleaved poly (ADP-ribose) polymerase; **GAPDH**, glyceraldehyde-3-phosphate dehydrogenase; **Ser**, serine; **Thr**, threonine.

nab-Sirolimus potentiated the cytotoxicity of fulvestrant

- In HR+, *PI3K*-mutated BrCa cell lines, the addition of 20 nM or 80 nM *nab*-sirolimus to fulvestrant significantly decreased cell viability (**Figures 2A** and **2C**) and nearly doubled cell death (**Figures 2B** and **2D**)

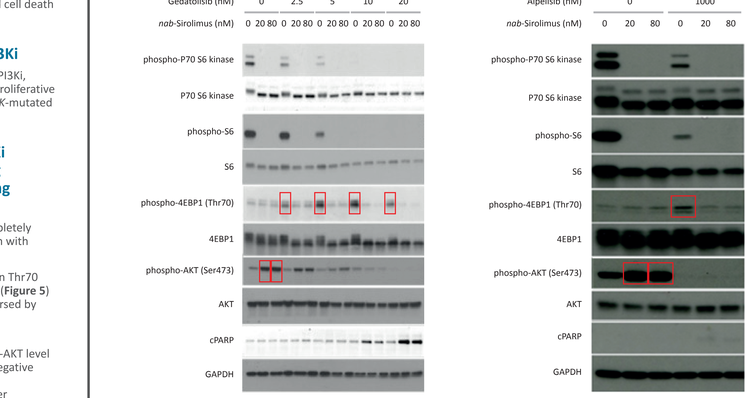
nab-Sirolimus in combination with a PI3Ki

- The addition of 20 nM or 80 nM *nab*-sirolimus to a PI3Ki, gedatolisib or alpelisib, significantly enhanced antiproliferative effects and increased cell death in HR– and HR+ *PI3K*-mutated BrCa cell models (**Figure 3**)

nab-Sirolimus plus fulvestrant or a PI3Ki provided synergistic effects by reducing compensatory activation of key signaling molecules

- Phosphorylation of p70 S6 kinase and S6 were completely eliminated by *nab*-sirolimus alone or in combination with fulvestrant (**Figure 4**) or a PI3Ki (**Figure 5**)
- Phosphorylation of the key mTORC1 target 4EBP1 on Thr70 was upregulated by fulvestrant (**Figure 4**) or a PI3Ki (**Figure 5**) alone in a dose-dependent manner, which was reversed by the combination with *nab*-sirolimus
- The phospho-AKT level was constitutively high in both HR+ and HR– *PI3K*-mutated cells; the phospho-AKT level was further increased by *nab*-sirolimus through a negative feedback loop, which was inhibited by the addition of fulvestrant or a PI3Ki in a dose-dependent manner (**Figures 4** and **5**)

Figure 5. Western blot analysis demonstrating PI3K/AKT/mTOR pathway crosstalk in HR–, HER2–, *PI3K*-mutated BrCa cells (MDA-MB-453)



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