Mutations in *PIK3R1* activate multiple targetable pathways in triple-negative breast cancer

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Abstract

Background: Approximately 5% of triple-negative breast cancers (TNBCs) carry mutations in the PIK3R1 gene (*PIK3R1*^{MUT}). Our prior studies showed that breast cancer cells lacking PIK3R1 protein had increased MEK activation, enhancing their responsiveness to the MEK inhibitors trametinib and binimetinib. Alpelisib targets the PI3K pathway. We hypothesized that alpelisib might also inhibit growth of *PIK3R1*^{MUT} TNBC. This study tested binimetinib, alpelisib, and their combination in PDX models with *PIK3R1*^{MUT}.

Methods: Five TNBC PDX models harboring either *PIK3R1*^{wt} or *PIK3R1*^{MUT} were studied (Table 1). Animals were treated with vehicle, binimetinib (10 mg/kg BID), alpelisib (35 mg/kg QD), or both for 28 days. Tumor volumes were recorded biweekly. All PDXs harbored *PIK3CA*^{wt} but had mutations in other PI3K or MAPK genes. Mass spectrometry was used to elucidate downstream protein and phosphoprotein expression.

Results: Single-agent binimetinib or alpelisib did not result in significant tumor growth reduction in most models harboring wild type *PIK3R1*. Binimetinib alone resulted in a slightly significant effect in Model C (not shown). A highly significant effect of both single agents was observed in the model harboring mutant PIK3R1 (p=0.0001). Binimetinib and alpelisib in combination significantly reduced tumor growth in wild type *PIK3R1* models (p=0.0126-0.0011); however, in the model harboring mutant *PIK3R1*, the most significant effect was observed (p<0.0001). Mass spectrometry analysis identified several pathways of interest, as described.

Conclusions: Mutations in *PIK3R1* sensitize TNBCs to combined MEK and PI3K pathway inhibition. *NF1* mutations alone do not confer sensitivity to single agents but enhance response when combined with binimetinib and alpelisib. These findings support the combination of MEK and PIK3CA inhibitors as a potential strategy for treating *PIK3CA*^{MUT} TNBCs.

Introduction

Breast cancer is the most prevalent cancer among women worldwide (1). While significant research has focused on the dysregulation of the PI3K signaling pathway in human breast cancers, most studies have concentrated on *PIK3CA*, the gene that encodes the catalytic subunit of the PI3K complex. Mutations in *PIK3CA* occur in 30-50% of breast cancers, leading to activation of the p110 protein, a key product of *PIK3CA* (2-4). However, fewer studies have examined the role of *PIK3R1*, which encodes the regulatory subunit (p85) of the PI3K complex. In previous work, we found that breast cancer cells lacking functional *PIK3R1* exhibited elevated levels of phosphorylated MEK, making them more sensitive to the MEK inhibitor, trametinib (5). This finding was observed in non-malignant isogenic breast cell lines lacking the *PIK3R1* gene. Additionally, we analyzed a cohort of ~4,300 human breast cancer cases and found that mutations or abnormal copy numbers of *PIK3R1* occur in about 2% of all breast cancers and 6% of triple-negative breast cancers (TNBC; 6). Building on this, we studied *PIK3R1* wild-type and mutant PDX models and showed that mutants are highly sensitive to the combination of alpelisib and binimetinib compared with wild type (7). In this study, we wanted to understand resistance mechanisms of cells surviving these drugs. We evaluated via proteomic methods the resistance to alpelisib, a p110 inhibitor, and binimetinib, a newer generation MEK inhibitor, both individually and in combination, in TNBC PDX models with either wild-type (wt) or pathogenic mutations (mut) in *PIK3R1*.

METHODS: Tumor extracts (pooled for each treatment group) were subjected to trypsinization and unique isobaric (TMT) labeling, using standard methods, combined into a single lysate and resolved into 15 fractions via RP-HPC. Each resulting fraction was subjected to phosphopeptide enrichment using High-Select TiO2 kit (Thermo). The resulting flow-through (unmodified peptides) and eluate (phosphopeptides) were then profiled (separately) using a Q Exactive HF mass spectrometer coupled with an UltiMate 3000 RSLC nanosystem with a Nanospray Frex Ion Source (Thermo Fisher Scientific). MaxQuant and Scaffold DDA software (v6.0.1) packages were then used to transform the raw counts into label ratios sorted by unique gene symbols. Label ratios were then used to define differences observed across tumor and treatment types.

Acknowledgements

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Results

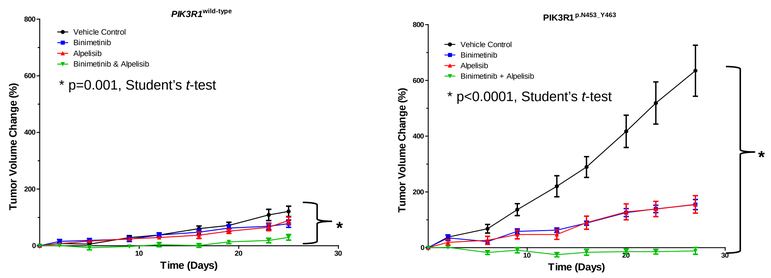


Figure 1. Representative Survival Curves. Impact of Alpelisib, Binimetinib, or Combination Treatment on TNBC; *PIK3R1*^{wt}; left panel) and TNBC *PIK3R1*$^{p.Asp569_Tyr580del}$; right panel). Mice were treated with vehicle control, 10 mg/kg single agent binimetinib po BID, or 35 mg/kg alpelisib po QD, or a combination of both treatments. Mice were dosed for 28 days. Student's *t*-test compares vehicle to combination treatment.

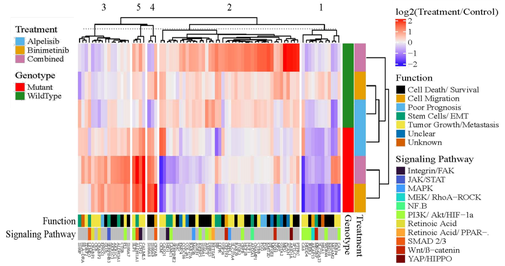


Figure 2. Heatmap of Proteins Identified via Mass Spectrometry. Heatmap illustrating differences in protein levels observed across treatment types (alpelisib, binimetinib, or combination treatment) in both wildtype and *PIK3R1* mutant tumor xenografts.

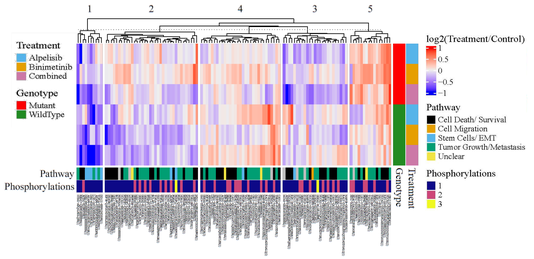


Figure 3. Heatmap of Phosphopeptides Identified via Mass Spectrometry. Heatmap illustrating differences in phosphopeptide levels normalized to corresponding non-phosphorylated peptides observed across treatment types (alpelisib, binimetinib, or combination treatment) in both wildtype and *PIK3R1* mutant tumor xenografts.

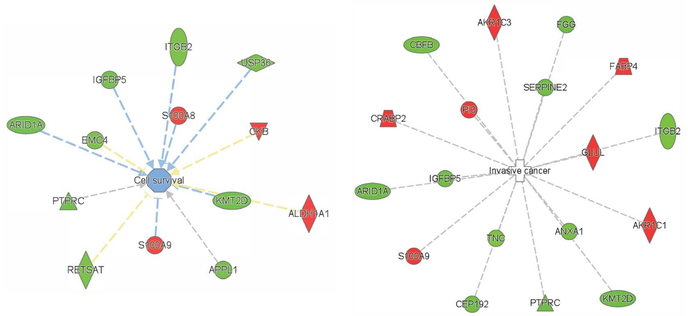


Figure 4. Network Maps based on Differential Protein Observations. Illustrations created in the Ingenuity Pathway Analysis Suite showing enrichments within the Cell Survival (left) and Invasiveness (right) pathways for the combined (alpelisib and binimetinib) treatments in the *PI3KR1* mutant tumor xenograft model, relative to wildtype.

Results

Animal Studies

Model PDX-wt (TNBC; *PIK3R1*^{wt}; Figure 1-left panel) responded slightly to the combination treatment. However, Model PDX-mut (TNBC; *PIK3R1*$^{p.Asp569_Tyr580del}$; Figure 1-right panel) responded to all (Alpelisib and/or Binimetinib) treatments, with the combination treatment resulting in extreme response.

Proteomic Analysis of Tumors

Quantitative mass spectrometry was used to assess protein levels in tumors lysates obtained from the animal studies at the 28-day timepoint. This analysis revealed modulation of multiple biological pathways associated with tumor growth, invasiveness/metastasis, and therapy resistance in the *PIK3R1* mutant samples.

1. **Untreated Tumors**: The differential analysis of observed proteins from the Vehicle Control treated samples (*PIK3R1* mutant vs. wildtype) suggest that the untreated mutant tumor has a highly aggressive, mesenchymal phenotype that is linked to cancer stem cell characteristics (e.g., higher levels of S100P; Versican (VCAN); Laminins (LAMA1-C1); proteases (e.g., KLK6/7), likely resulting in enhanced invasive/metastatic behavior, and higher proliferative potential.
2. **Treatment with PIK3CA (Alpelisib) and/or MEK (Binimetinib) Inhibitors:** Multiple synergistic effects were observed in several cellular processes (relative to wildtype). Below, we detail a selection of the modulations observed in surviving, excised (resistant) tumors after 28-days of treatment, with an emphasis on tumor resistance to combination therapy.

   Calcium Signaling, Motility, and Metastasis: Differential gene expression analysis revealed a dramatic upregulation of key calcium-signaling proteins, including Calmodulin (CALM1) and S100 Calcium-Binding Protein A4 (S100A4), under combined treatment unique to the *PIK3R1* mutant tumors. This suggests an enhanced calcium-mediated signaling response in the resistant mutant cells, likely driving increased cell motility, invasion, and survival pathways, conferring a drug-resistant, metastatic-like phenotype.

   Cancer Stem Cell Differentiation and Apoptosis Resistance: Upregulation of aldehyde dehydrogenases (e.g., ALDH1A1, ALDH3A1, AKR1C1/3) was observed almost exclusively in the *PIK3R1* mutant tumors following combined treatment. High ALDH expression is a hallmark of Cancer Stem Cell (CSC) differentiation and resistance to chemotherapy. This upregulation suggests a mechanism by which mutant cells survive drug-induced oxidative stress and DNA damage, evading apoptosis, and that surviving, resistant cells may be sensitive to ALDH inhibitors.

   Chromatin Remodeling and Transcriptional Control: The observed inability to upregulate ARID1A, a subunit of the SWI/SNF (BAF) chromatin remodeling complex, was unique to the *PIK3R1* mutant tumors with combination treatment. This is expected to result in repressed transcriptional and epigenetic regulation in the mutant cells that silences tumor-suppressor genes and activates pro-survival oncogenic programs. Notably, ARID1A is also frequently mutated in breast cancers, and clinical trials are exploring the efficacy of ARID1A inhibition.

   Neutrophil Recruitment: The combination treatment resulted in a synergistic reduction in Neutrophil recruitment exclusively within the *PIK3R1* mutant tumors, as evidenced by lower levels of multiple murine neutrophil biomarkers (e.g., MPO, CYBB, NCF1) in tumors treated with combination treatment vs. vehicle control.

Conclusions

In advanced cancers, compensatory resistance mechanisms to targeted therapy are usually inevitable. This study identified multiple potential resistance pathways to combination PI3K and MEK inhibition in TNBC that were distinct, based on *PIK3R1* mutational status. This knowledge may improve our ability to apply adjunctive treatment strategies, in *PIK3R1*–mutant cancers to enhance therapeutic efficacy of combined PI3K and MEK inhibition.

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