The Oncologist
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Supplemental Appendix for:
KRAS-mutated, ER-positive low-grade serous ovarian cancer Unraveling an exceptional response mystery
Shumei Kato et al.

Appendix S1. Supplemental Methods

Patient and Molecular Testing

The patient was consented on IRB approved UCSD-PREDICT study (NCT02478931) to publish clinical information in this article. The patient was evaluated for clinical characteristics and outcome of intervention. Tumors were provided as formalin-fixed, paraffin-embedded (FFPE) samples and a 315 gene panel was evaluated for mutations by NGS performed in a Clinical Laboratory Improvement Amendments (CLIA)-certified lab (Foundation Medicine, Cambridge, MA) [24]. Blood-derived cell-free DNA (cfDNA) was evaluated by NGS for the presence of mutations in 73 genes by Guardant 360 (http://www.guardant360.com/). ER positivity by immunohistochemistry was defined as ≥1+ and ≥10% of cells stained.

Cell Culture Materials and Reagents

OVCAR3, OVCAR5 and SKOV3 cells were purchased from the National Cancer Institute (NIH). Cell line models were cultured in RPMI 1640 supplemented with FBS (10%), penicillin (100 unit/ml), streptomycin (100 µg/ml), and l-glutamine (2 mm) at 37 °C in 5% CO2. For hormone depletion, cells were plated at 30% confluence in phenol red-free medium supplemented with charcoal-stripped FBS and incubated at 37 °C in 5% CO2 for 48 h prior to experimental procedures. 17-β-estradiol (2824), Trametinib (10399) and tamoxifen (6342) were purchased from Tocris Biosciences (Minneapolis, MN). Erlotinib was purchased from Sigma-Aldrich (St. Louis MO). Culture media was changed to phenol-red free media with charcoal stripped FBS 48 hours prior to cell treatment to strip cells of hormone. Cells were then either treated with compounds at indicated doses within each figure for 48 hours. Following treatments cells were either analyzed by MTT proliferation assays or prepared for Western blot analysis.

We utilized tamoxifen and the MEK inhibitor trametinib in our studies. The clinical effects of letrozole come from the inhibition of aromatases that produce estrogen, most of which are found in peripheral and adipose tissues. Cancer cell lines, in contrast, only have a limited ability to produce estradiol. We therefore utilized hormone stripped media in our experiments to mimic the conditions that would be anticipated in vivo upon letrozole treatment, and we supplemented media with estradiol to mimic the conditions that would be expected in vivo in the absence of letrozole treatment. With this experimental approach, we were poised to probe how RAS and ER signaling pathways together influence cellular proliferation.

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Proliferation assays

Cells (5000 per well) were seeded in 96-well plates in phenol red–free medium supplemented with charcoal-stripped FBS. Treatments were initiated after the cells were attached. At the 48 hour time point, cell viability was determined by MTT assay (3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide); 10 μl of MTT (5 mg/ml in phosphate-buffered saline) was added to each well followed by incubation at 37°C for 2 hours. The formazan crystal sediments were dissolved in 100 ul of dimethyl sulfoxide, and absorbance was measured at 590 nm using a Tecan Infinite 200 PRO plate reader. Each treatment was performed in eight replicate wells and repeated three times.

Western Blot Analysis

Cell lysates were generated using radioimmunoprecipitation assay buffer [150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM tris (pH 8.0)] containing protease inhibitor cocktail (Cell Signaling Technology) and incubated on ice for 1 hour. The total protein concentration was determined by Pierce Protein assay (Thermo Fisher Scientific). Protein samples (20 μg) were resolved by electrophoresis on 12% SDS–polyacrylamide gels and electro- phoretically transferred to polyvinylidene difluoride (PVDF) mem-branes (Millipore Corporation) for 20 min at 25 V with trans-blot turbo station (Biorad). The blots were probed with the appropriate primary antibody and the appropriate fluorophore-conjugated secondary antibody. The protein bands were visualized using the Licor CLx Odyssey imaging station (Licor Biosystems).

Statistical analysis

All statistical analysis were carried out using Graphpad Prism 8 software. One-way ANOVA with post-hoc Tukey's test was used to calculate statistical significance. A P-value of less than 0.05 was considered to be significant.