Supplemental material

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Cell lines

The cell lines KGN, COV434, A549 (lung adenocarcinoma) and MIA PaCa2 (pancreas carcinoma) were used.

The KGN cell line (Nishi et al, 2001) originating from a recurrent AGCT was confirmed to harbor the FOXL2 c.402C>G mutation. The COV434 cell line (Van den Berg-Bakker, et al, 1993) which originates from a juvenile GCT, does not harbor the FOXL2 mutations and lacks also FOXL2 protein expression. Both cell lines were obtained from Riken BioResource Center. The KGN, COV434, A549 and MIA PaCa2 cells were cultured in DMEM supplemented with 10% FBS, and penicillin/streptomycin.

Single-drug treatment

We tested the viability of the four cell lines in response to the treatment with four different drugs: ketoconazole, medroxyprogesterone, abiraterone and letrozole. For that purpose, five thousand cells per well were seeded in black walled-micro-clear bottom 96-well plates (Greiner 655986). 24h later we started the treatment, that was maintained during 72h. For the treatment, we prepared one-third dilutions of the drugs (nine in total), starting with 100uM.

After 72h of incubation, the cell viability was measured using the Cytell Cell Imaging System, after staining with Hoesht 33342 (Life Technologies #H3570) (cell-permeable nuclear marker) and Propidium Iodide (Sigma #P4864) (cell impermeable nuclear marker). This assay allowed us to

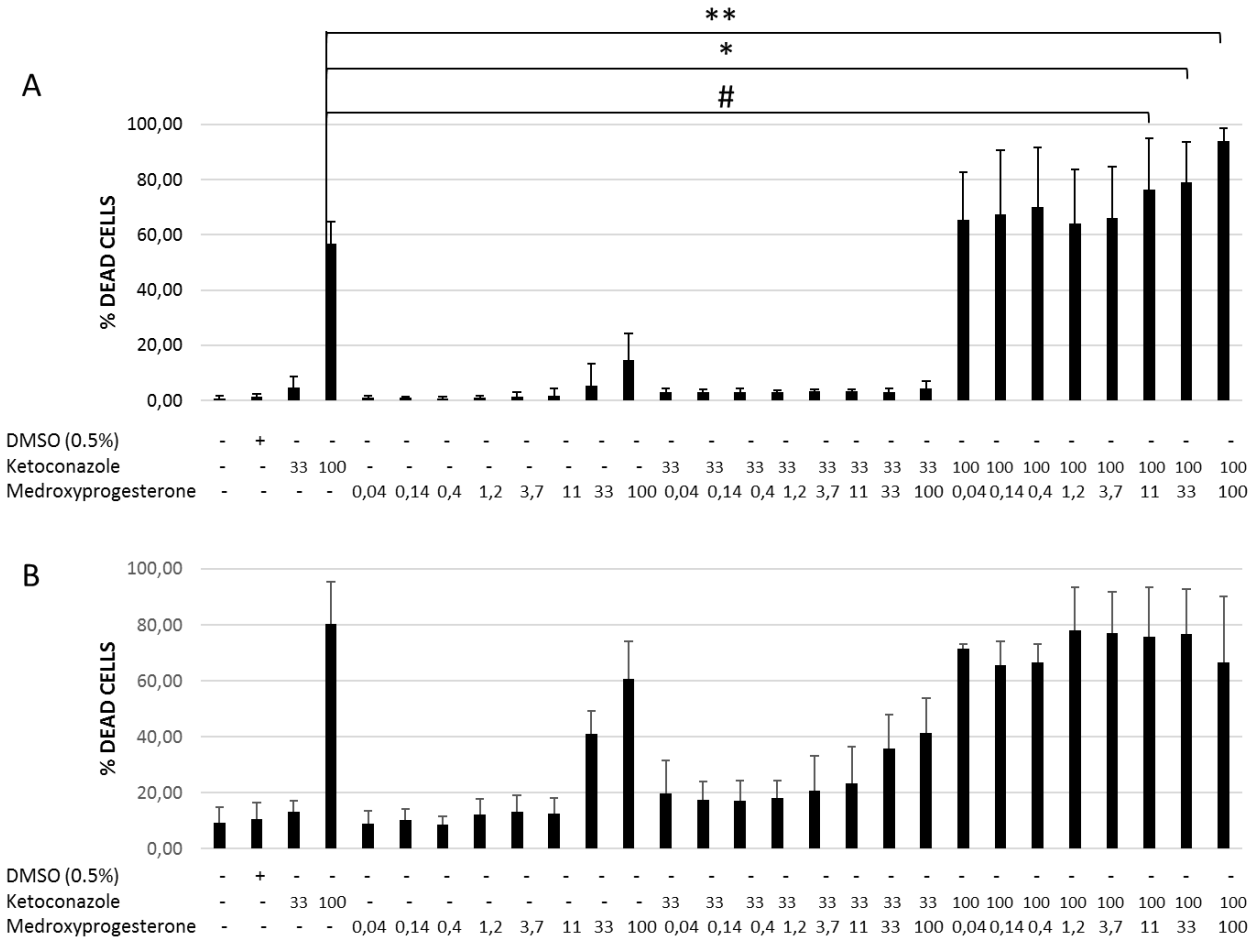
determine the number and percentage of live and dead cells in each well. Each experiment was done in triplicates. (Figure 4 in the main text)

Drug combination treatment

The cell viability of KGN and COV434 cells was tested in response to the combination of ketoconazole and medroxyprogesterone. For that purpose, five thousand cells were seeded in Black walled-micro-clear bottom 96-well plates (Greiner 655986). 24h later we started the treatment, that was maintained during 72h. For the treatment, we prepared one-third dilutions of the medroxyprogesterone (eight in total), starting with 100uM, that were combined with ketoconzale 33 or 100uM.

After 72h of incubation, the cell viability was measured using the Cytell Cell Imaging System, as described above.

Figure S1: Effect of the combination ketoconazole plus medroxyprogesterone in KGN and COV434 cells



KGN (A) and COV434 (B) cells were treated with different concentrations of medroxyprotesterone alone or in combination with ketoconazole (33 or 100uM),. Viability was measured 72h after exposure to the drugs by Cytell Cell Imaging system

(a) The combination of ketoconazole 100uM and medroxyprogesterone (11, 33 and 100uM) showed an additive effect in KGN cells in terms of cell death induction. Graphs represent the percentage of dead cells, as mean of three independent experiment ± standard deviation.

(b) The combination of both drugs didn't produce additive effect in COV434 cells. Graphs represent the total number of cells respect to the control (mean of three independent experiments ± standard deviation)

*, $p<0.01$; **, $p<0.0001$; #, $p<0.05$

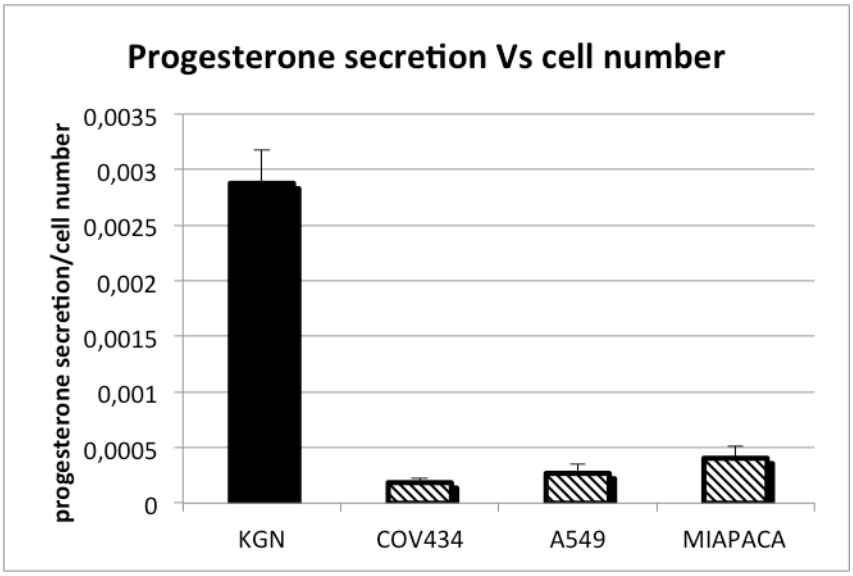
Progesterone secretion measurement

In order to confirm hormonal overproduction in GCT the cell lines KGN, COV434, A549 and MIA PaCa2 cells were seeded in 24-well plates (100.000 cells/well) in DMEM supplemented with 10%FBS. After 24h of incubation, we changed the medium to DMEM without phenol red supplemented with 10% of charcoal stripped FBS.

After 48h of incubation, we measured the levels of progesterone in the supernantant of the cell culture. The attached cells were collected and counted in a flow cytometer, in order to normalize the levels of progesterone by number of cells. Each treatment was done in triplicate.

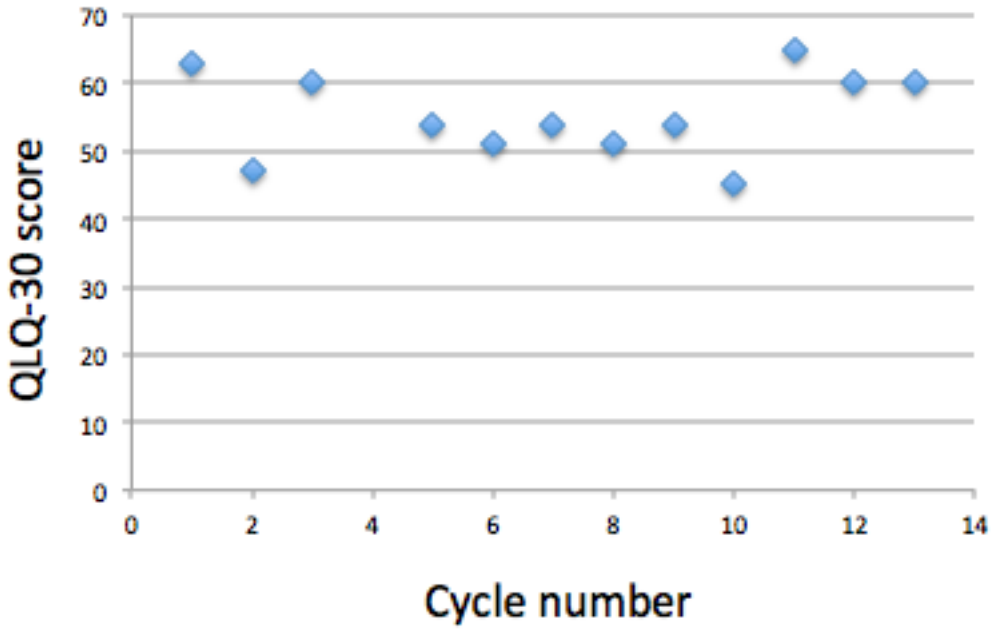
The determination of the levels of progesterone was done using the Progesterone Enzyme Immunoassay kit (Arbor Assays, Ann Arbor, Michigan, USA), following the protocol of the manufacturer. The analysis of the data was done using the online tool from MyAssays (http://www.myassays.com/arbor-assays-progesterone-eia-kit.assay).

Figure S2: Hormonal (progesterone) secretion in cell lines

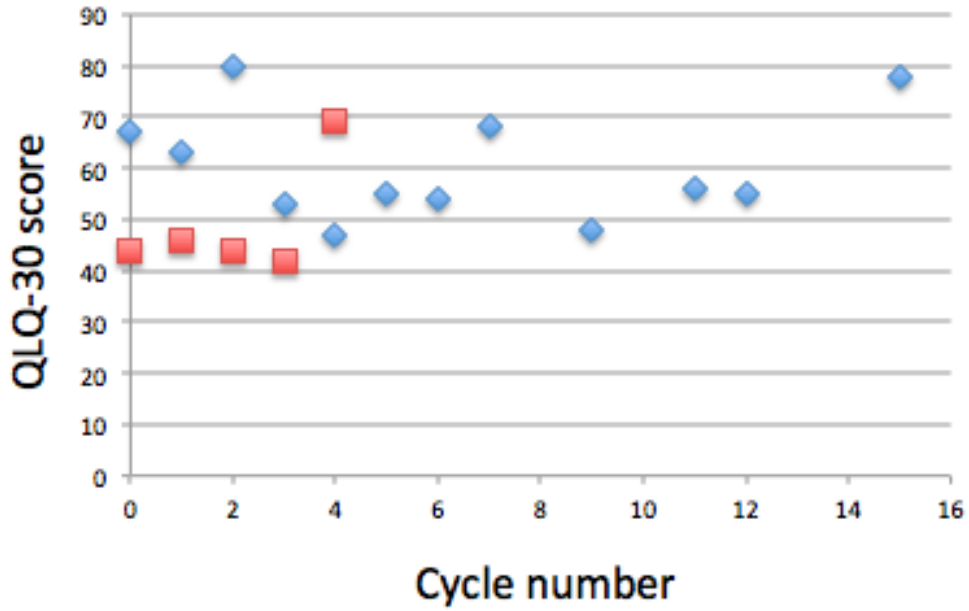


KGN, COV434, A549 and MiaPaca2 cells were culture in DMEM without phenol red supplemented with 10% charcoal stripped FBS. 48h later the secretion of progesterone was measured in the supernatant using the Progesterone Enzyme Immunoassay kit (Arbor Assays, Ann Arbor, Michigan, USA). The graph represents the levels of progesterone relative to the number of cells.

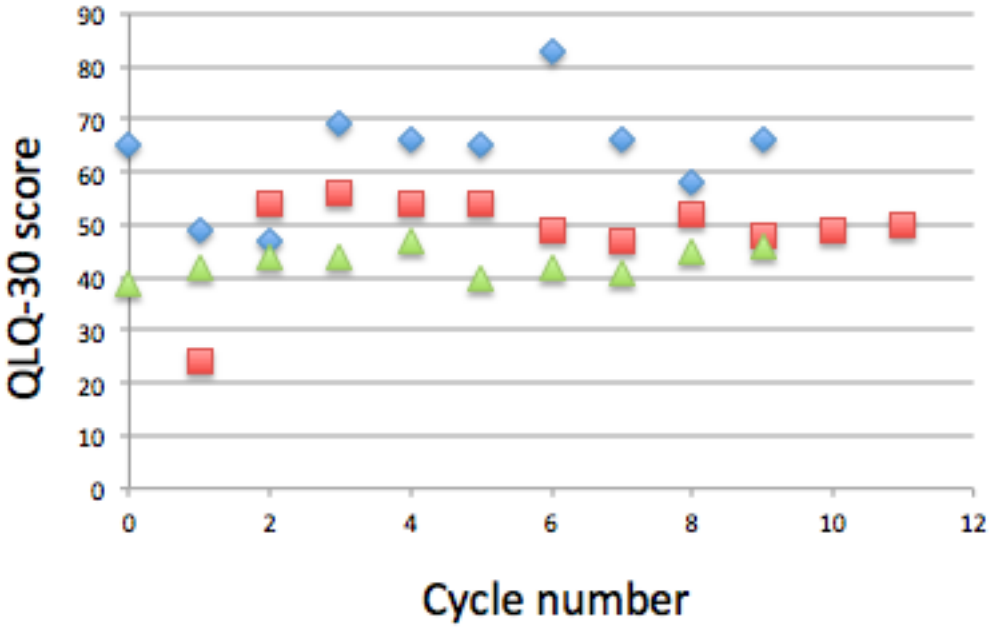
Figure S3. Evolution of QoL (EORTC QLQ-C30)



A. Patient with unknown FOXL2 status (case 3).



B. Patients without FOXL2 mutation: case 1 (red square) and 2 (blue diamond).



c. Patients with confirmed FOXL2 mutation. case 4 (green triangle), 5 (red square) and 6 (blue diamond).