Title page

Open label phase III clinical trial of ketoconazole as CYP17 inhibitor in metastatic or advanced non-resectable granulosa cell ovarian tumors. The GREKO (GRanulosa Et KetOconazole) trial. GETHI 2011-03

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Abstract (240)

Background: Granulosa cell ovarian tumor (GCT) is characterized by a pathognomonic mutation in the FOXL2 gene (402 C>G) that leads to an overactivation of steroidogenesis. CYP17 is a key enzyme in such process and can be inhibited by ketoconazole.

Methods: We designed a phase III clinical trial to assess the efficacy of ketoconazole in advanced GCT and conducted several in vitro studies to support the clinical findings.

Results: From October 1st 2012 to January 31st 2014, 6 evaluable patients were recruited in ten hospitals of the Spanish Group for Research in Orphan and Unfrequent Tumors (GETHI). FOXL2 (402C>G) mutation was confirmed in three; two cases were wild type and it could not be assessed in one.

No objective response by RECIST was observed but five cases achieved stable disease longer than 12 months. Median progression free survival was 14·06 months (CI95% 5,43 to 22,69) for the whole study population (3·38 and 13·47 months for wild type cases and 14·06, 20,67 and 26,51 for those with confirmed FOXL2 mutation). Median overall survival was 22·99months (CI95% 8·99 to 36·99).

In vitro assays confirmed the activity of ketoconazole in this tumor and suggested potential synergisms with other hormonetherapies.

Conclusion: Ketoconazole has shown activity in advanced GCT in clinical and in vitro studies. Based on these data an orphan designation was granted by the European Medicines Agency for ketoconazole in GCT (EU/3/17/1857).

ClinicalTrials.gov Identifier: NCT01584297

Body text (2700)

Introduction

Granulosa cell ovarian tumor (GCT) is an infrequent subtype of cancer well differentiated from epithelial tumors. It accounts for 5% of all ovarian malignancies and, with an incidence of 0.4-1.2 cases per 100000 habitants, is considered a rare disease. (Orphanet ID: ORPHA99915)[1]

Although most cases are identified at initial stages, and can be cured through surgical resection, distant recurrences have been documented even 10 years after resecting the primary tumor. Unfortunately, at advanced stage, it is a lethal condition.[2]

Because of the low incidence of this tumor, randomized clinical trials are lacking and current evidence for treatment is limited to case reports, retrospective studies and some phase II clinical trials where different types of sex cord ovarian (SCO) cancers were allowed.[3-12]

The most remarkable characteristic of this disease is the presence, in around 94% of cases, of a single punctual mutation in the gene FOXL2 [402C→G (C134W)] (OMIM: 605597).[13] FOXL2 physiologically downregulates the transcription of the gene STAR (that encodes the STeroidogenic Acute Regulatory Protein) and CYP17 through a direct interaction with the steroidogenic factor-1 (SF-1). Preclinical models have shown that mutations of the FOXL2 protein could disrupt its union with SF-1, enhancing the expression of CYP17 by 5 folds.[14]

CYP17 is the enzyme that transforms pregnelonone and progesterone in their 17-hidroxilated forms. Additionally it converts 17-OH-progesterone in androstendione, from which all other androgens are derived. Based on the key role of CYP17 in androgen synthesis, ketoconazol (a strong inhibitor of CYP17) has been used in the last decades in prostate cancer achieving biochemical and radiological responses.[15]

Additionally, mutant FOXL2 presents and enhanced affinity for the aromatase promoter (also known as CYP19) that is responsible for progesterone synthesis downstream CYP17. [16,17]

With the support of the Spanish Group for Research in Orphan and Unfrequent Tumors (GETHI) we designed a multicenter phase III clinical trial to assess the efficacy and tolerability of ketoconazole in advanced GCT.

Methods

Patients

Key eligibility criteria were an age of 18 years or older, histologically confirmed GCT, radiologically measurable lesions not amenable to radical surgical resection, an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1 and an adequate hematologic and biochemical function.

Study design

This study was an independent investigator-initiated, open-label, phase III clinical trial that was conducted in 10 institutions in Spain. The collaborative group "Spanish Group for Research in Orphan and Unfrequent Tumors" (GETHI) sponsored the study and disseminated it among all its members in order to enhance recruitment. Funding was provided by the Spanish Ministry of Health 2011 call for independent clinical research (file number EC11-178).

Ketoconazole was administered at a standard dose of 400 milligrams every 8 hours per day on a 28- day cycles. Concomitant oral hydrocortisone (30 mg at breakfast and 20mg in the evening) was administered as prophylaxis of suprarenal insufficiency following clinical practice.

The trial conformed to the principles of the Declaration of Helsinki and the Good Clinical Practice Guidelines and was approved by the ethics committee at each study center and by the Spanish regulatory agencies. (NCT01584297; EudraCT No: 2012-001948-21). All patients gave written informed consent.

Only investigators participating in the trial were involved in the design of the trial, the analysis of the data, and the writing of the manuscript. None who is not an author contributed to the preparation of the manuscript.

Study Assessments

Patients were seen at the start of every treatment cycle for physical examination, determination of ECOG performance status, and a complete blood count and serum biochemical measurements. Tumor response, measured according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1, was assessed every 8 weeks by means of thoracic and abdominal computed tomography. All radiological studies were reviewed by a central radiologist, blinded to clinical data and patient outcome. Safety assessments were performed before each treatment cycle following the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Endpoints

The primary endpoint of the study was to determine the efficacy of ketoconazole in terms of overall response rate (ORR) in metastasic or locally advanced non-resectable granulosa cell ovarian cancer, measured by a centralized radiologist.
Secondary objectives were safety profile, progression free survival (PFS) assessed by the central radiologist and local investigators, quality of life (assessed with the questionnaire EORTC QLQ-C30) and Overall survival (OS).

Statistical Analysis

Sample size was calculated using the two stages Gehan´s model. Using this method it was estimated that 17 patients had to be included in the first stage in order to demonstrate treatment efficiency of at least 10%. The sample size calculation was made based on the following parameters, type I error probability $\alpha = 0.05$, test power $(1 - \beta) = 0.8$. A detailed description of the statistical plan can be found in the protocol (attached).

PFS and OS were analyzed with the Kaplan-Meier method. Adverse events were described according to the treatment period, with the omission of deaths from progression of granulosa cell carcinoma.

Correlative studies

Since this is a rare disease, tumor samples from all cases were required to be reviewed

centrally by an anatomopathologist specialized in gynecological tumors and unaware of the clinical evolution, response to treatment or mutational status.

The presence of the pathognomonic *FOXL2* (402C→G) mutation was assessed from genomic DNA isolated from each fixed and formalin paraffin-embedded (FFPE) tissue block (5 5 m-thick sections) and subsequent PCR using primers encompassing the *FOXL2* mutation. PCR amplicons were purified and sequenced by Sanger methodology.[18]

In vitro assays

In order to support clinical results with in vitro experiments the cell line named KGN, an adult GCT model that harbors the FOXL2 (402C→G) mutation, was studied. The cell lines COV434 (model of juvenile GCT without FOXL2 mutations but with protein downexpression), A549 (lung adenocarcinoma) and MIA PaCa2 (pancreas carcinoma), were used as controls (all technical details are provided as supplementary material).

Results

Patients and Administered Treatments

From October 1st 2012 to January 31st 2014, 7 patients were enrolled in the study that was stopped early because of low recruitment and a shortage of ketoconazole. One patient withdrew consent before taking any dose of treatment and was excluded of data analysis. Demographic and clinical characteristics of the patients are presented in **Table 1.** Toxicity was recorded on day one of every cycle.

As per protocol, patients experiencing clinical benefit as judged by her attending physician were permitted to continue on treatment beyond the initially scheduled six months.

FOXL2 mutation assessment

FOXL2 mutation could be assessed in five cases with three confirmed as positive and two as negative. Unfortunately pathological review could only be performed in the three positive cases, since tissue was scarce in the other two and completely

consumed in mutational analysis. (**Table 1**)

Efficacy

No objective response was seen. Five patients (83%) achieved disease stabilization for more than one year.

Median progression free survival was 14·062 months (CI95% 5,43 to 22,69) for the whole study population. (**Figure 1**)

Wild type cases achieved a PFS of 3·38 and 13·47 and mutated patients 14·06, 20,67 and 26,51 months. (**Figure 2**)

Median overall survival was 22·99 months (CI95% 8·99 to 36·99) for the whole study population. (**Figure 3A**)

Wild type cases achieved an OS of 6·47 and 37·62 months and mutated patients 20·67, 32·33 and 60·02 months. (**Figure 3B**). One patient (case 4) remains alive by the submission of this manuscript.

Safety

In total 27 adverse events were reported. Three reached grade 3 but only one, pneumonia, was deemed as possibly related to study drug by the local investigator (**Table 2**).

No death, treatment reduction or withdrawal was due to toxicity.

Quality of life

No relevant changes were observed in QoL of patients included along the study (**Fig S3**)

In vitro studies

Ketoconazole and abiraterone, both CYP17 inhibitors, showed significant effects on cell viability, in terms of increased cell death, in KGN and COV434, while no effects were observed in A549 or MIA PaCa2 cells (**Fig. 4A**).

Ketoconazole showed to be the most potent drug in KGN and COV434 cells, with percentages of cell death around 80 and 100% respectively at a concentration of 100uM.

However, ketoconazole, medroxyprogesterone and abiraterone produced significant decreased in the total number of cells, indicating a reduced growth rate in all the cases (**Fig. 4B**).

The combination of ketoconazole (100uM) and medroxyprogesterone (33 or 100uM) showed a synergistic effect on cell death in KGN cells but not in COV434. (**Fig. S1**)

Finally, we confirmed progesterone overproduction, in KGN cell culture compared to controls (**Figure S2**).

Discussion

We present the results of a clinical trial focused in granulosa cell ovarian cancer that assessed the efficacy of ketoconazole, a CYP17 inhibitor, in advanced disease. GCT is a rare entity that represents a unique model of cancer with a "monomutational" origin. The FOXL2 [402C→G (C134W)] mutation is considered pathognomonic and alternative diagnosis must be considered when deemed as negative in tumor samples.[19,20] One of the most interesting consequences of such alteration is the overexpression of the CYP17 and CYP19 enzymes that lead to steroid hormones overproduction including estrogens and androgens. Additionally, androgen and progesterone receptors are both overexpressed in GCT supporting a potential role for hormonal treatments in this tumor.[21] CYP17 is a targetable enzyme and its inhibition has shown to be highly efficacious in hormone sensitive tumors as prostate cancer. New generation inhibitors, as abiraterone, have also reached the market and many others are under development.[22] With this molecular background in mind and the positive experience of a single case previously treated with ketoconazole by our group (already communicated) we decided to perform a clinical trial with such drug, the oldest and most accessible CYP17 inhibitor, in GCT.[23]

Since this is a rare disease the support of a big collaborative group (the Spanish Group for Research in Orphan and Infrequent Tumors [GETHI]) was required in order to disseminate the study and enhance recruitment. Additionally, as an orphan indication

with little commercial interest, funding was provided by a public institution (the Spanish Ministry of Health) through the 2011 call for grants for independent research. Despite this remarkable effort and although ten institutions all over the country agreed to take part in the trial, only seven cases could be included. The low recruitment rate and a ketoconazole shortage in 2014, led to the decision of early terminating the trial.

Low recruitment is a common problem in infrequent entities and a major issue when trying to incorporate new treatments to GCT. In fact, all previous trials allowed the inclusion of different subtypes of SCOs.

In 1999 two clinical trials established the activity of cisplatin based chemotherapy, that became the standard in advanced stages. Unfortunately, the design of these studies allowed heterogenous populations and mixed the evaluation of systemic treatments and surgical procedures .[4,5]
More recently, paclitaxel was deemed as ineffective in a phase II trial where 31 SCOs achieved a median PFS of 10.0 months.[12] Finally, bevacizumab monotherapy led to a PFS of 9.3 months in 36 cases.[13]

In our study only GCT cases were eligible. Though no objective response was observed, up to 83% of patients reached a disease stabilization longer than one year. Notably, median PFS (14 months) and OS (23 months) were similar to some historical data achieved with cisplatin based chemotherapy but with no relevant toxicity.[4] In fact, the only grade 3 adverse event deemed as related to ketoconazole, pneumonia, could likely be explained by the advanced tumor disease than by the drug.

An important issue when working with rare diseases is to ensure diagnostic accuracy. It has been communicated that around 21% of cases of GCT need to be reclassified when reviewed by expert pathologists.[24]

Since multiple centers participated in this trial and many patients were referred from institutions not directly involved in the study, central review of the samples, was

considered key. Additionally we decided to assess the presence of the FOXL2 mutation 402C>G in tumor tissue not only by its diagnostic value but by its relevance in the rational of the study. Unfortunately molecular analysis could be performed only in five cases and pathological examination in three. Of interest, all cases confirmed as granulosa tumors by the central pathologist presented the FOXL2 mutation and reached long PFS and OS.

Finally, to better support the clinical findings, we studied the effects of ketoconazole in two cell cultures (KGN and COV434) largely used as GCT models. As described in the text, KGN is an adult type granulosa tumor and has been confirmed to harbor the FOXL2 mutation in heterocigosity. COV434 is a juvenile GCT where FOXL2 is wild type but the expression of the gene is abolished. In both cases ketoconazole showed to be the most potent drug, in terms of cell death induction, compared to other hormonal agents like medroxiprogesterone and letrozole.

In conclusion, our results seem to point out CYP17 inhibition as a promising strategy for GCT that warrants further study. Importantly, based on these data the European Medicines Agency granted an orphan designation for ketoconazole in GCT (EU/3/17/1857).

As continuation of this work the GETHI has launched two additional trials, named GREKO II and III, assessing the efficacy of Orteronel and enzalutamide respectively. Results from these studies could definitively help to elucidate the role of homonetherapy in this rare disease.

Figures/Tables (4)

Table 1. Study population characteristics and outcome

Case #	Age (years)	#Prior lines of treatment	Metastasic sites	FOXL2 status	Central pathologic review	PFS (months)	Reason for treatment cesation	Best radiological response
1	61	3	Peritoneum	wild type	NA	3,38	PD	SD
2	44	3	Peritoneum, bone, liver	wild type	NA	13,47	PD	SD
3	63	5	Peritoneum, lung	NA	NA	22,6	PD	SD
4	60	1	Nodes, lung	Mutant	Confirmed	14,06	PD	SD
5	45	3	Peritoneum, pelvis	Mutant	Confirmed	20,67	PD	SD
6	59	1	Peritoneum, spleen, lung	Mutant	Confirmed	26,51	PD	SD

NA: tissue Not Available for mutation study or central pathologic review; PD Progressive disease; SD: Stable Disease

Table 2. Secondary effects

GRADE 1-2 ADVERSE EVENTS	N(%)
ASTHENIA	6 (100)
ANOREXIA	2(33)
DISFONIA	3(50)
MUCOSITIS/BUCAL INFECTION	3(50)
VOMITING	3(50)
DIAORRHEA	4(66)
CONSTIPATION	1(16)
CEPHALEA	1(16)
GGT ELEVATION	2(33)
HYPERBILIRRUBINEMIA	1(16)
ALOPECIA	1(16)
SKIN RASH	3(50)
EDEMA	4(66)
PAIN	4(66)
HYPERTENSION	1(16)
COUGH	1(16)
PERIPHERAL NEUROPATHY	3(50)
RENAL INSUFICIENCY	1(16)
TREMOR	1(16)
FEVER	1(16)
RESPIRATORY INFECTION	2(33)
URINARY INFECTION	1(16)
URINARY INCONTINENCE	1(16)
METRORRAGY	1(16)
GRADE 3 ADVERSE EVENTS	
ALLERGIC REACTION	1(16)
PNEUMONIA	1(16)
HYPERGLUCEMIA	1(16)

Figure 1. Progression Free Survival (months) for the whole study population

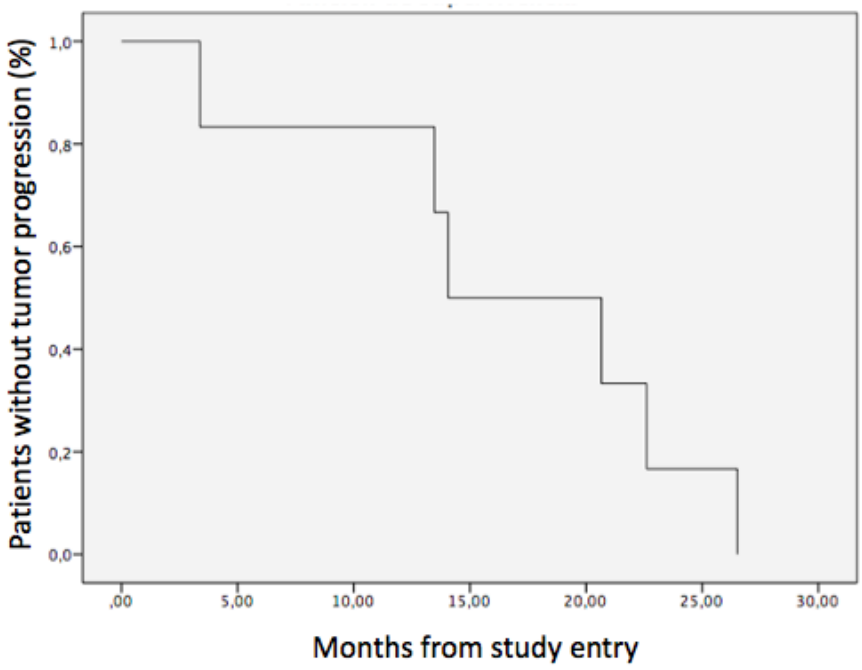
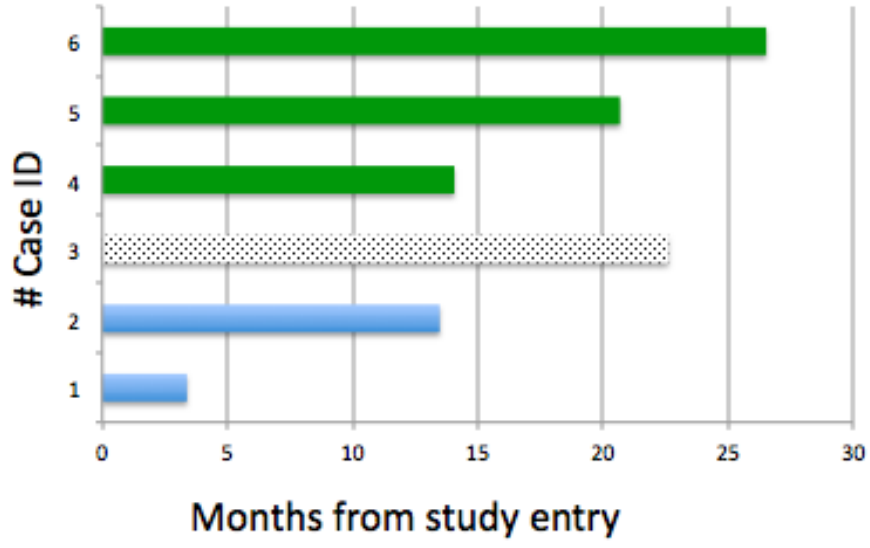
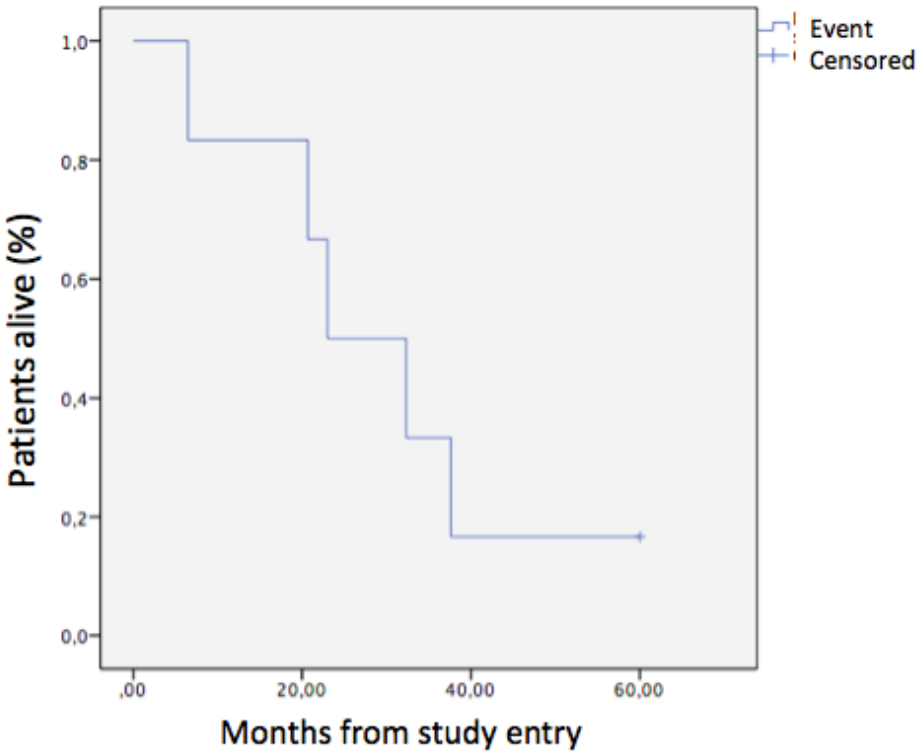


Figure 2. Progression Free Survival (months) case by case

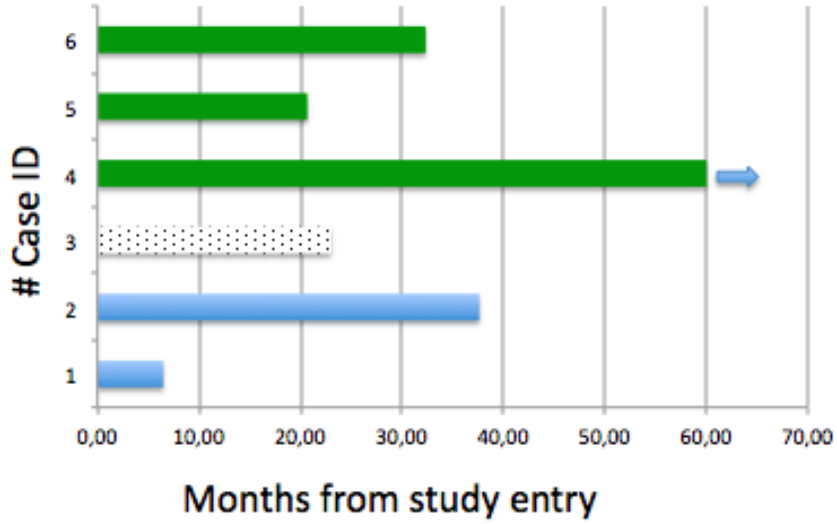


Mutated cases: green bars; wild type cases: blue bars; case with mutational status Not Assessed: doted bar.

Figure 3. Overall Survival (months)

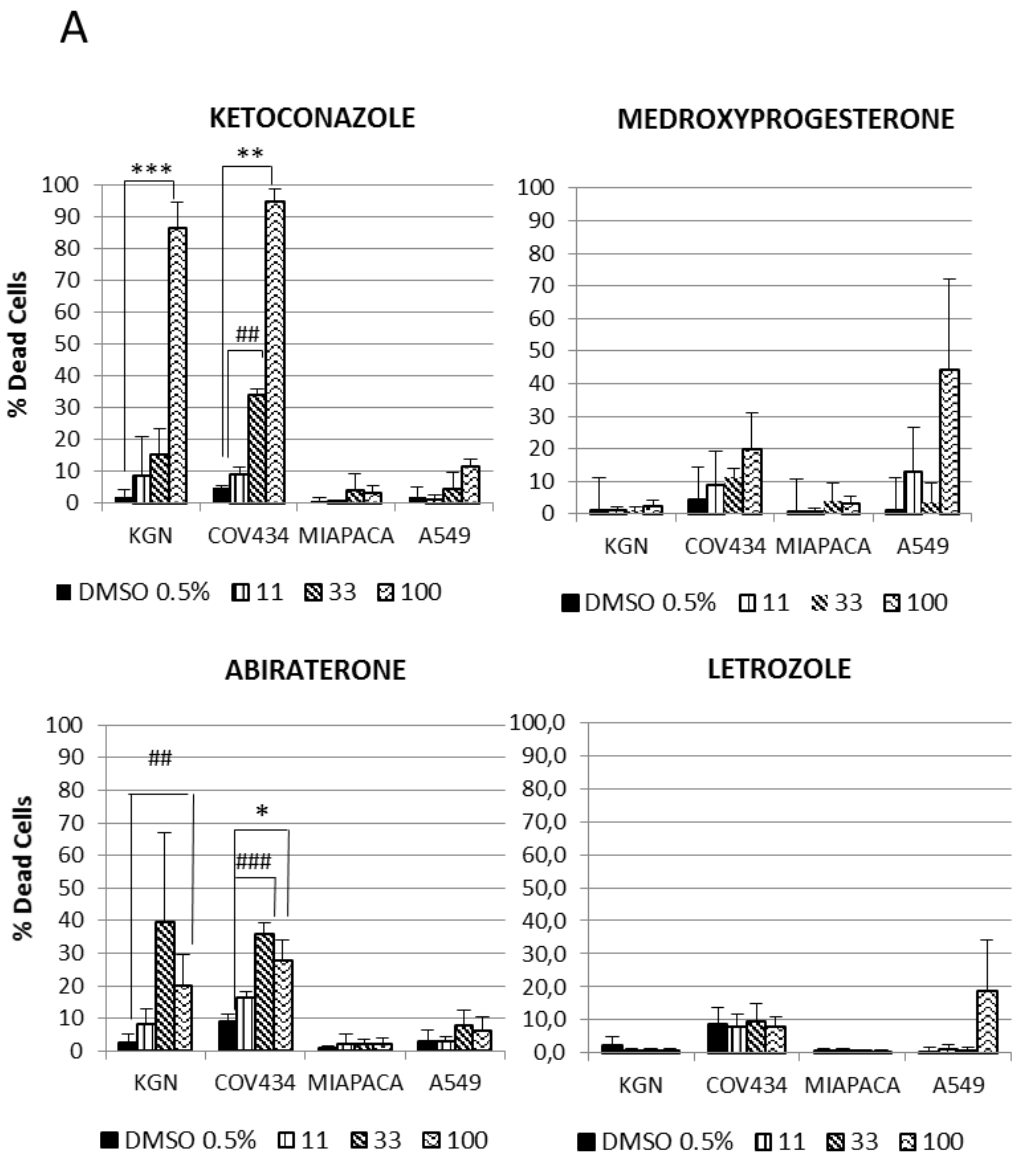


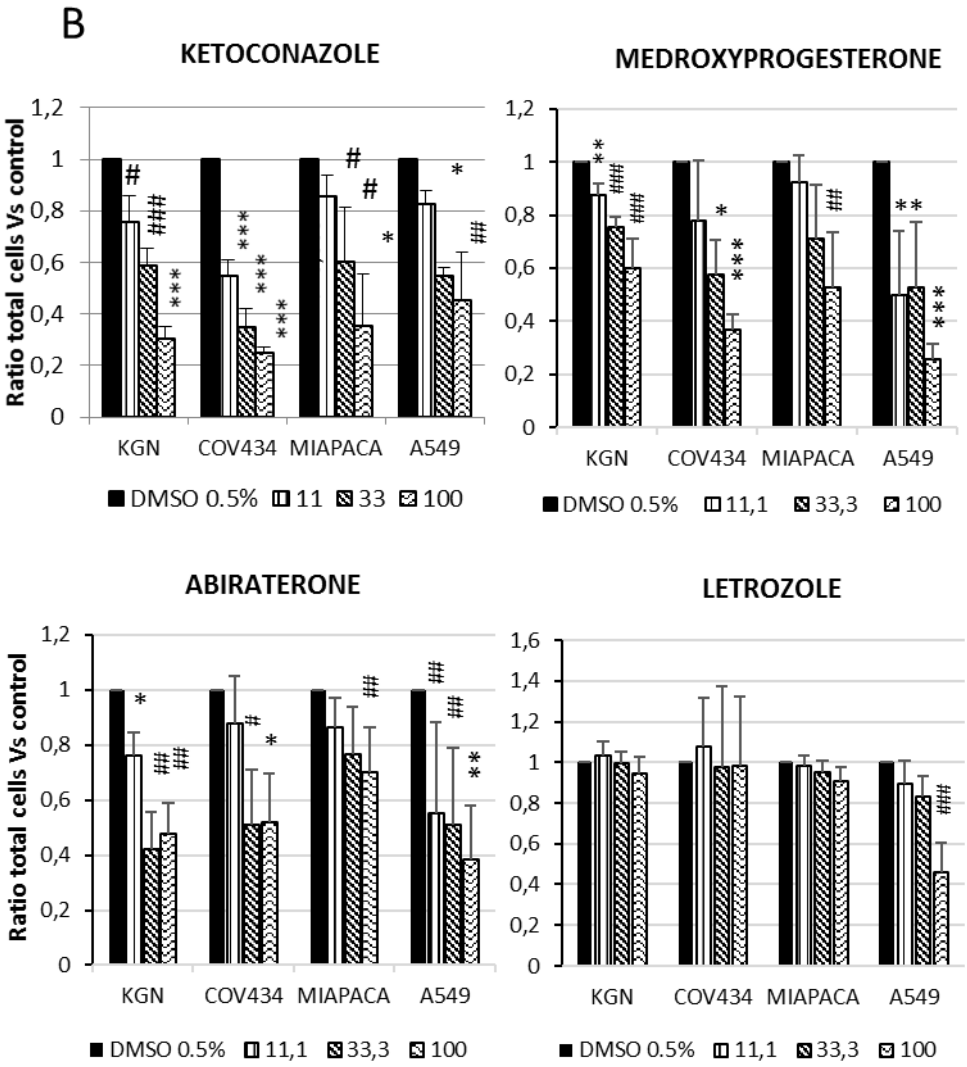
A. Whole study population



B. Overall Survival (months) case by case
Mutated cases: green bars; wild type cases: blue bars; case with mutational status Not Assessed: doted bar. Blue arrow: patient alive

Figure 4. Viability assays in response to different hormonetherapies





KGN, COV434, A549 and MIA PaCa2 cells were treated with different concentrations (11, 33 or 100uM) of ketoconazole, medroxyprogesterone, abiraterone or letrozole. Viability was measured 48h after exposure to the drugs by Cytell Cell Imaging system.

A. Ketoconazole and abiraterone induce cell death in granulosa cell lines. Graphs represent the percentage of dead cells, as mean of three independent experiment ± standard deviation.

B. Ketoconazole, medroxyprogesterone and abiraterone stop the growth of all the cell lines. Graphs represent the total number of cells respect to the control (mean of three independent experiments ± standard deviation)
*, p<0.01; ** ,p<0.001; ***, p<0.0001; #, p<0.05; ##, p<0.005; ###, p<0.0005

Supplemental material

Index

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

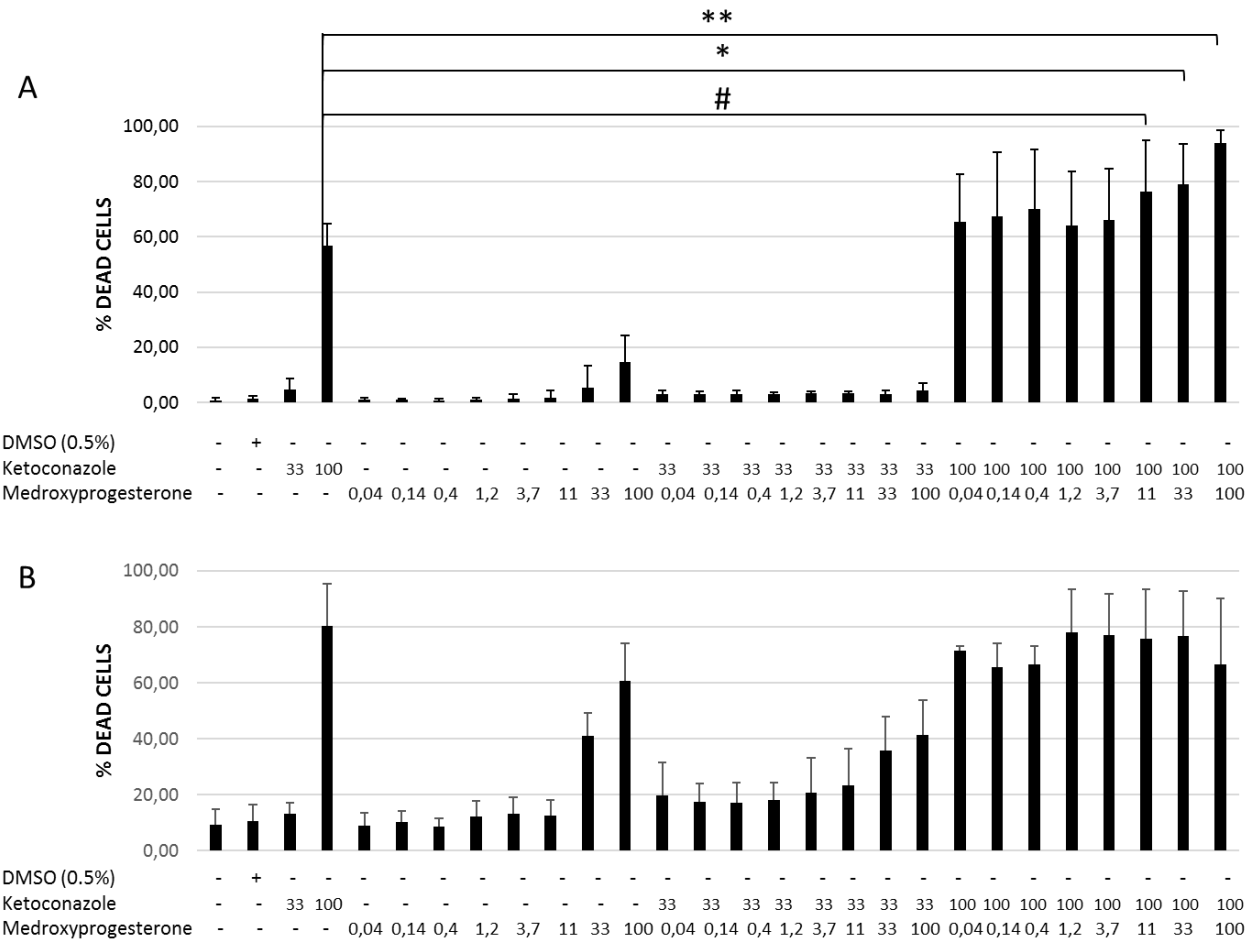
dead cells in each well. Each experiment was done in triplicates. (Figure 4 in the main text)

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)

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

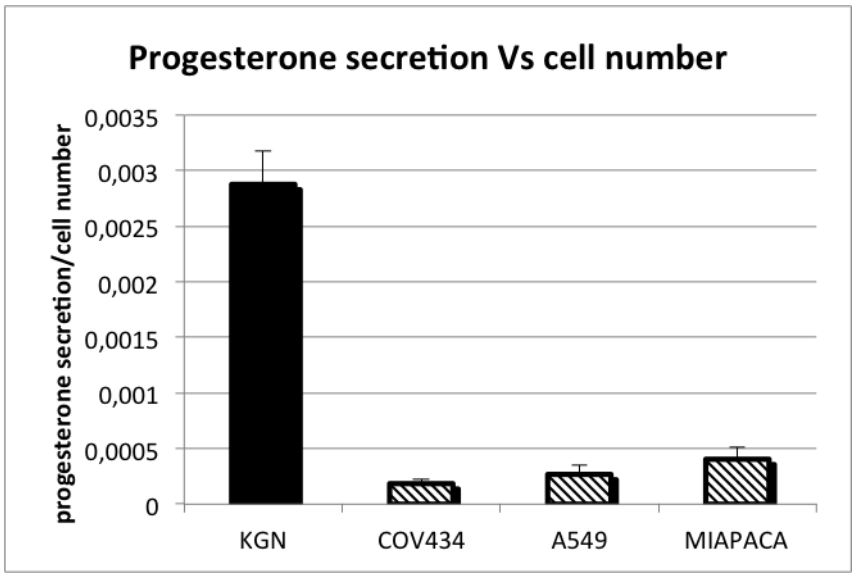
Progesterone secretion measurement

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.

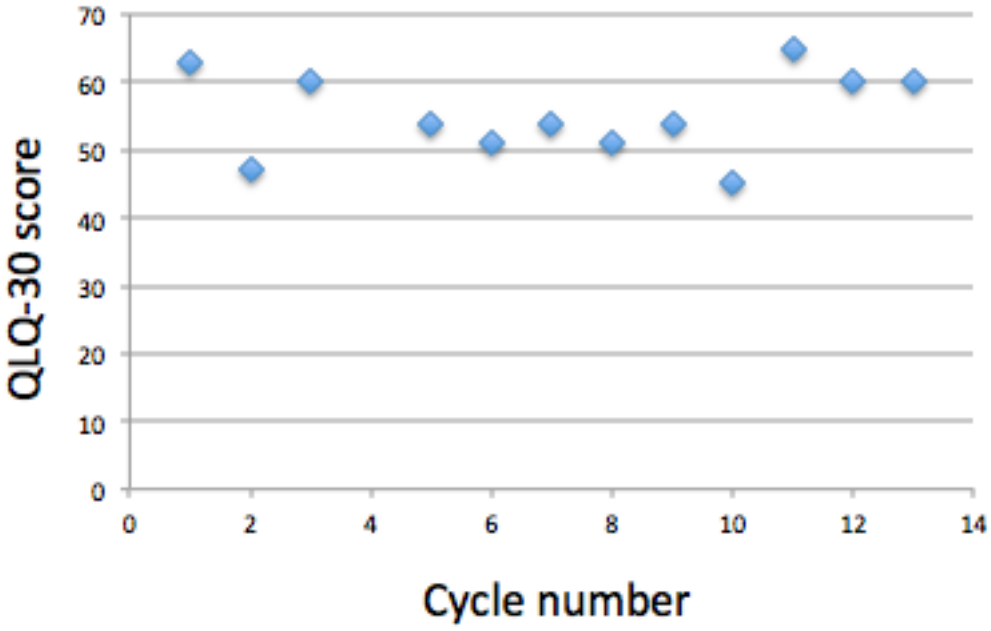
The determination of the levels of progesterone was done using the Progesterone Enzyme Immunoassay kit (Arbor Assays, Ann Arbor, Michigan, USA), following the protocol of the manufacturer. The analysis of the data was done using the online tool from MyAssays (http://www.myassays.com/arbor-assays-progesterone-eia-kit.assay).

Figure S2: Hormonal (progesterone) secretion in cell lines

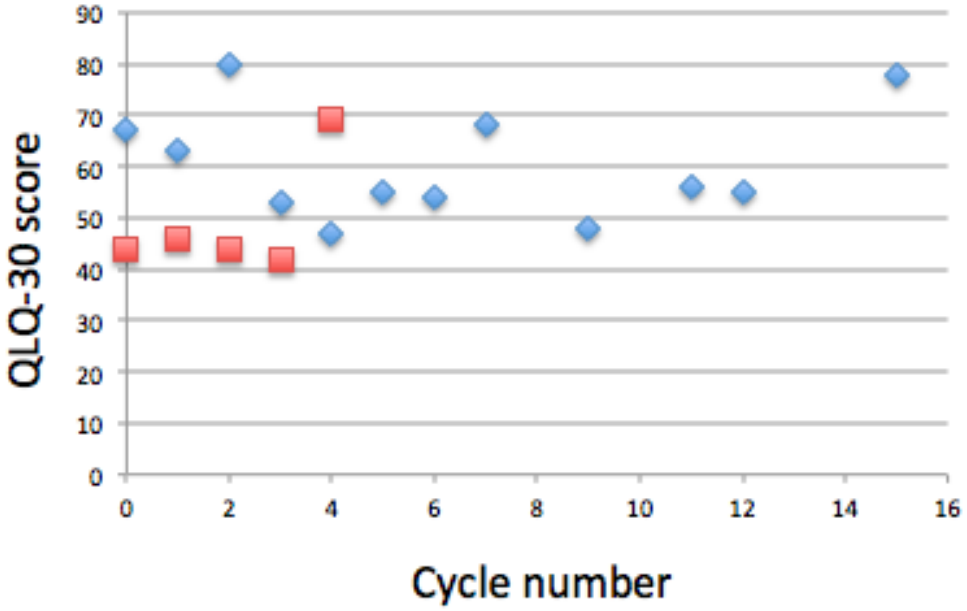


KGN, COV434, A549 and MiaPaca2 cells were culture in DMEM without phenol red supplemented with 10% charcoal stripped FBS. 48h later the secretion of progesterone was measured in the supernatant using the Progesterone Enzyme Immunoassay kit (Arbor Assays, Ann Arbor, Michigan, USA). The graph represents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

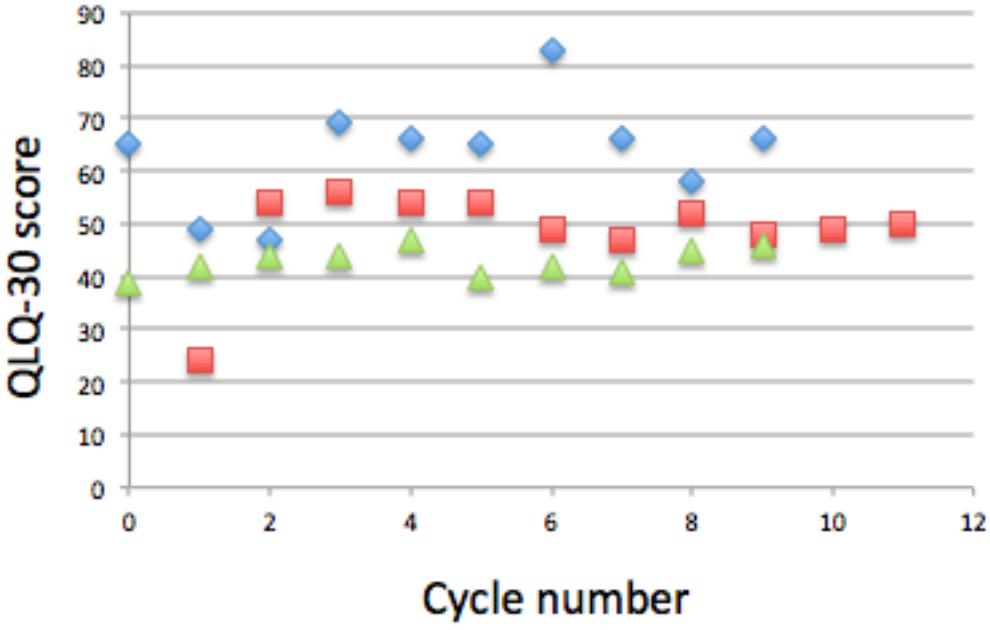
Figure S3. Evolution of QoL (EORTC QLQ-C30)



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).

Author contribution

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References (24)

[1] Schumer ST, Cannistra SA. Granulosa cell tumor of the ovary. J Clin Oncol 2003;21:1180-9.

[2] Mangili G, Ottolina J, Gadducci A, et al. Long-term follow-up is crucial after treatment for granulosa cell tumours of the ovary. Br J Cancer 2013;109:29-34.

[3] Homesley HD, Bundy BN, Hurteau JA, Roth LM.Bleomycin, etoposide, and cisplatin combination therapy of ovarian granulosa cell tumors and other stromal malignancies: A Gynecologic Oncology Group study. Gynecol Oncol. 1999 Feb;72(2):131-7.

[4] Pecorelli S, Wagenaar HC, Vergote IB et al. Cisplatin (P), vinblastine (V) and bleomycin (B) combination chemotherapy in recurrent or advanced granulosa(-theca) cell tumours of the ovary. An EORTC Gynaecological Cancer Cooperative Group study. Eur J Cancer. 1999 Sep;35(9):1331-7.

[5] Fishman A, Kudelka A, Edwards C et al. GnRH (Depot-Lupron) in the treatment of refractory or persistent ovarian granulosa cell tumors (GCT). Proc Am Soc Clin Oncol 1994, 13:236 (abst).

[6] Isaacs R, Forgeson G, Allan S. Progestagens for granulosa cell tumours of the ovary. Br J Cancer. 1992 Jan;65(1):140.

[7] Malik ST, Slevin ML. Medroxyprogesterone acetate (MPA) in advanced granulosa cell tumours of the ovary--a new therapeutic approach? Br J Cancer. 1991 Mar;63(3):410-1.

[8] Martikainen H, Penttinen J, Huhtaniemi I, Kauppila A. Gonadotropin-releasing hormone agonist analog therapy effective in ovarian granulosa cell malignancy. Gynecol Oncol. 1989 Dec;35(3):406-8.

[9] Hardy RD, Bell JG, Nicely CJ, Reid GC. Hormonal treatment of a recurrent granulosa cell tumor of the ovary: case report and review of the literature. Gynecol Oncol. 2005 Mar;96(3):865-9.

[10] Fishman A, Kudelka AP, Tresukosol D et al. Leuprolide acetate for treating refractory or persistent ovarian granulosa cell tumor. J Reprod Med. 1996 Jun;41(6):393-6.

[11] Burton ER, Brady M, Homesley HD, et al. A phase II study of paclitaxel for the treatment of ovarian stromal tumors: An NRG Oncology/ Gynecologic Oncology Group Study. Gynecol Oncol. 2016 Jan;140(1):48-52.

[12] Brown J, Brady WE, Schink J, et al. Efficacy and safety of bevacizumab in recurrent sex cord-stromal tumors: results of a phase 2 trial of the Gynecologic Oncology Group. Cancer. 2014 Feb 1;120(3):344-51.

[13] Shah SP, Köbel M, Senz J, et al. Mutation of FOXL2 in granulosa-cell tumors of the ovary. N Engl J Med. 2009 Jun 25;360(26):2719-29.

[14] Park M, Shin E, Won M, et al. FOXL2 Interacts with Steroidogenic Factor-1 (SF-1) and Represses SF-1-Induced CYP17 Transcription in Granulosa Cells. Mol Endocrinol. 2010 May;24(5):1024-36.

[15] Speight JL, Roach M, 3rd. Advances in the treatment of localized prostate cancer: the role of anatomic and functional imaging in men managed with radiotherapy. J Clin Oncol 2007;25(8):987.

[16] Fleming NI, Knower KC, Lazarus KA, Fuller PJ. Aromatase is a direct target of FOXL2: C134W in granulosa cell tumors via a single highly conserved binding site in the ovarian specific promoter. PLoS One. 2010 Dec 20;5(12):e14389.

[17] Belli M, Iwata N, Nakamura T, Iwase A, Stupack D, Shimasaki S. FOXL2C134W-Induced CYP19 Expression via Cooperation With SMAD3 in HGrC1 Cells. Endocrinology. 2018 Apr 1;159(4):1690-1703.

[18] Rosario R, Wilson M, Cheng WT, Payne K, Cohen PA, Fong P, Shelling AN. Adult granulosa cell tumours (GCT): clinicopathological outcomes including FOXL2 mutational status and expression. Gynecol Oncol. 2013 Nov;131(2):325-9.

[19] Schrader KA, Gorbatcheva B, Senz J et al. The specificity of the FOXL2 c.402C>G somatic mutation: a survey of solid tumors. PLoS One. 2009 Nov 24;4(11):e7988.

[20] Jamieson S, Butzow R, Andersson N et al. The FOXL2 C134W mutation is characteristic of adult granulosa cell tumors of the ovary. Mod Pathol. 2010 Nov;23(11):1477-85. doi: 10.1038/modpathol.2010.145.

[21] Maise Al Bakir, Lei Wang, Kenneth Russell, et al. Molecular analysis of non-epithelial ovarian cancer by histologic subtype.J Clin Oncol 32:5s, 2014 (suppl; abstr 5570).

[22] Ryan CJ, Smith MR, Fizazi K et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 2015 Feb;16(2):152-60.

[23] Garcia-Donas J, Hurtado A, García-Casado Z, et al. Cytochrome P17 inhibition with ketoconazole as treatment for advanced granulosa cell ovarian tumor. J Clin Oncol. 2013 Apr 1;31(10):e165-6.

[24] Maillet D, Goulvent T, Rimokh R, et al. Impact of a second opinion using expression and molecular analysis of FOXL2 for sex cord-stromal tumors. A study of the GINECO group & the TMRO network. Gynecol Oncol. 2014 Jan;132(1):181-7.