Ovarian adult-type granulosa cell tumor: focusing on endocrine-based therapies

Annie D Yang¹, John Curtin¹ & Franco Muggia^{*,1}

¹NYU School of Medicine & Divisions of Medical Oncology & Gynecologic Oncology of the Perlmutter Cancer Center at NYU Langone Health, New York, NY 10016, USA

*Author for correspondence: franco.muggia@nyumc.org

Practice points

- FOXL2 mutation is diagnostic, and, if pathology is in doubt, it should be performed.
- Surgical resection and staging is key to assess prognosis.
- Platinum-based adjuvant chemotherapy such as with carboplatin plus paclitaxel is a reasonable step for women diagnosed with distant recurrences or poor prognostic initial features. However, evidence for improved outcome is lacking.
- Hormone-based therapies, especially with aromatase inhibitors, have shown activity and future studies may lead to further insight on the relationship of inhibition of hormonal pathways and tumor progression.

Adult-type granulosa cell tumors (GCTs), although rare, are the most commonly diagnosed neoplasms arising in the endocrine-active ovarian stroma. They are characterized by excessive production of estrogens, antimüllerian hormone and inhibins. In 2009, a specific mutation in *FOXL2* was identified to be pathognomonic of GCTs. How dysregulation of this transcription factor, resulting in upregulation of aromatase, leads to unchecked proliferation, and progression to a malignancy, remains unclear. The key pathological and clinical feature of GCTs that affects their usually favorable outcomes is a diagnosis of greater than Stage 1 disease at presentation. Chemotherapy is given as adjuvant upon an advanced stage diagnosis; however, its effect on survival upon recurrence is modest. On the other hand, aromatase inhibitors also lead to tumor regression and are suitable for long-term maintenance.

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Adult-type granulosa cell tumors (GCTs) are hormone-producing tumors of the granulosa cell layer of the ovary, making up 2–5% of all malignant ovarian neoplasms [1]. The median age of presentation is around 50, with the majority of patients being diagnosed in Stage I because of symptoms related to excess estradiol production and abnormal uterine bleeding [2,3]. In addition to estradiol, determinations of inhibin B and antimüllerian hormone (AMH) levels are helpful in diagnosis and in monitoring disease progression when there is dissemination. With the 2009 discovery of the *FOXL2* mutation responsible for disease pathogenesis, a role for hormonally based therapies in the treatment of GCT has been sought [4]. In this review, we highlight the emerging role of aromatase inhibitors (AIs) to treat the disease when it is metastatic and their potential in building future therapeutic strategies.

Normal physiology

GCTs are derived from the granulosa cell layer of the ovarian follicle. Normally, these cells proliferate in response to rising circulating gonadotropins and decline in response to circulating testosterone [5]. The granulosa cells and theca cells are part of the ovarian follicle and form the cellular stroma that surrounds the developing oocyte. They normally function to produce estradiol via a two-cell mechanism: the theca cells convert cholesterol to androstenedione and testosterone and the granulosa cells convert androstenedione and testosterone into estradiol. Granulosa cells, when

stimulated by follicle-stimulating hormone (FSH), drive the conversion of testosterone to estradiol via aromatase CYP19A1 [6]. Excess estradiol, when sensed by the hypothalamus and anterior pituitary, decreases the production of gonadotropin-releasing hormone (GnRH), FSH and LH [7]. Stimulation of granulosa cells by activin increases FSH binding and FSH-induced aromatization in the follicles. It also enhances LH's action on the theca cells of the ovaries to enhance androgen synthesis. Granulosa cells also produce AMH and inhibins A and B. Inhibin B is produced as a result of FSH stimulation and provides feedback to the pituitary to decrease the production of FSH.

Pathogenesis of hormone overproduction by GCT & identification of FOXL2 mutation

Excess estradiol produced by the tumor often results in abnormal uterine bleeding, postmenopausal bleeding and abdominal pain [2,3]. In women who still possess a uterus, the excess estradiol from GCT leads to an increase in endometrial hyperplasia/carcinoma that is often diagnosed synchronously with the ovarian tumor [8]. Since the work of David Huntsman's group, the cause of excess steroidogenesis in GCTs has been linked to a somatic pathognomonic mutation of FOXL2 (C402G; C134W) that was identified in 2009 and initially found in 97% of GCT cases [9]. When this mutation is not found, the diagnosis is in doubt.

Wild type (wt) FOXL2 is a forkhead-helix transcription factor that normally directly upregulates aromatase (CYP19A1) production – an enzyme that catalyzes the last step of estrogen synthesis [10]. It is also required for folliculogenesis and maintenance of the adult ovary. wt-FOXL2 has diverse transcriptional activities including regulation of cell death, proliferation and tumorigenesis. FOXL2 C134W retains some of the same transcriptional activities of the wild-type allele but with subtle alterations in the expression of a set of proto-oncogenic genes [11].

In the adult ovary, FOXL2 plays a crucial role in regulating the production of steroid hormones. The wt-FOXL2 interacts with the DNA-binding domain of steroidogenic factor-1 (SF-1), thereby inhibiting the ability for SF-1 to transcriptionally activate the expression of CYP17, a key enzyme of androgen steroid synthesis that converts progesterone to 17-hydroxyprogesterone and then to androstenedione [12,13]. FOXL2 also antagonizes the CYP17 promoting activity of SF-1 by transcriptionally repressing SF-1 expression and antagonizing WT1 during mouse ovarian development [13,14]. Theca cells of the ovary are responsible for androgen synthesis and CYP17 expression, but not traditionally linked to granulosa cells [15]. This suggests that FOXL2 may play a role in suppressing theca-cell specific genes within granulosa cells. The expression of key enzymes in hormonal synthesis needs to be studied further in the presence of FOXL2 mutations.

In fact, other mutations in FOXL2 have been shown to interrupt wt-FOXL2's ability to inhibit SF-1, therefore leading to unrepressed expression of CYP17 and production of steroids [13]. The FOXL2-SF-1 complex has also been described to bind directly to the promoter of aromatase CYP19 and cell lines expressing mutant FOXL2 C134W have shown increased stimulation of aromatase CYP19 [10,16]. Although deregulation of the expression of CYP17 as a result of the FOXL2 C134W mutation has not been confirmed *in vitro*, it may serve as a target for future research because of the possibility that it might contribute to overproduction of sex steroids in GCTs. The steroidogenic nature and pathognomonic mutation make GCTs different from other ovarian neoplasms and raises many questions that remain unanswered such as whether beta estradiol and/or other hormones act via the stroma and/or its vasculature to contribute to malignant transformation. Moreover, how inhibiting hormone production relates to the antitumor activity in the presence of metastatic disease has been the subject of small series and anecdotal reports to be reviewed later.

In addition to synthesizing excess estrogen, GCTs also produce AMH and inhibin B. Both have been shown to be more accurately associated with disease presence and correlated to tumor size [17]. AMH is also regulated by SF1 and produced in the ovarian granulosa cells [18]. Inhibin B is produced normally in the granulosa cells in females in response to FSH from the anterior pituitary. Inhibin and estrogen act to downregulate the hypothalamic–pituitary–gonadal axis. Inhibin provides negative feedback to the anterior pituitary to specifically suppress FSH production by the basophilic cells [19]. By contrast, FSH production stimulates the conversion of testosterone to estradiol in the granulosa cells.

Pathology & clinical course

Initial findings on abdominal imaging often include an adnexal mass around 10 cm in size that is often both solid and cystic [20]. Histologically GCTs are typically characterized by Call-Exner bodies and coffee bean nuclei [21]. Surgical staging is the most important indicator of prognosis, recurrence, and treatment [1]. Small series have reported low recurrence rates in Stage I (5.4%) rising to 21.1 and 40% in Stages III and IV, respectively [22]. For the patients who are diagnosed at Stage Ic with excrescences on the surface of the ovary and at more advanced resectable

stages, adjuvant chemotherapy is usually offered [22,23,24]. GCTs are characterized by late recurrences after resection, sometimes as long as 20 years, but with subsequent increasing aggressiveness [25]. 5- and 10-year disease-free survivals are high (around 90%) for early stage disease [26,27]; however, once a patient recurs, the likelihood of death from disease rises [28]. In the recent National Cancer Database (NCD) study analyzing 2680 women registered from 1998 to 2013, the 5-year survival for Stage II–IV disease has been found to be 80.8% (75.7–86.3%), 77.4% (71.9–83.3%) and 54.6% (44.9–66.3%), respectively [27]. Due to poor prognosis upon recurrence and its indolent clinical course, recommendations often stress life-long follow-up and tumor marker screening. With identification of the current pathognomonic mutation, early identification of recurrence by circulating tumor DNA has been suggested [29]. Metastases occur mostly locally within the peritoneal cavity, but not infrequently also spreading to lungs, liver and spleen, and rarely to bone and brain; spread to regional lymph nodes is also rare but may occur more frequently in higher stage disease [22,26,27,30]. In the above database, 1346 reported on lymph node status, and only 42 were positive.

Treatment overview

Surgery

Surgery (unilateral oophorectomy) is the primary treatment for early stage GCT tumors [31]. Stage I (confined to the ovary) is the most common presentation and surgery is mostly curative. As with other tumors of mesenchymal origin, lymphadenectomy has not been considered to play a role in the management of these patients [26]. The yield of lymphadenectomy, as noted above, has been shown to be low. However, in the recent analysis of the NCD, completely surgically staged patients fared significantly better. Adjuvant therapies, traditionally chemotherapy, are given in cases of high stage disease (Stage Ic or higher) [24]. Since overproduction of estradiol from the tumor is a feature of granulosa cell tumors, accompanying uterine pathology is common. The process of addressing atypical hyperplasia or endometrial carcinomas subsequently would lead to computerized tomography, hysterectomy, and resection of a unilateral adnexal tumor. Inhibin B levels, if initially elevated, may be a helpful tumor marker for early detection of recurrence. Although often recommended after completion of adjuvant chemotherapy, it is unusual to continue such surveillance beyond annual intervals. Given the rarity of this tumor, such guidelines are individualized and not based on clinical trials.

Optimal debulking surgery is often performed and recommended in the treatment of disease recurrence. Chemotherapy is usually provided after any disease recurrence given the eventual high risk of mortality from tumor progression. Therefore, more frequent and more sensitive means of surveillance may be justified upon recurrence. Radiologic surveillance is performed before and after delivery of postoperative chemotherapy for recurrences and subsequent surveillance frequency can be adjusted to the aggressiveness of the disease. The use of positron electron tomography to help define malignant potential has not been studied.

Chemotherapy

The Gynecologic Oncology Group began studying chemotherapy for GCT in the 1970s; their focus was on combination chemotherapy regimens initially developed for other diagnoses. Details on these trials are not relevant to this review, but led to identifying a number of potentially active drugs. Nevertheless, when high risk features are identified in the surgical specimen or there is an advanced stage at presentation even if fully resected, adjuvant chemotherapy is usually provided. The most common regimens were initially borrowed from the treatment of germ cell tumors and consist of cisplatin plus etoposide, often with added bleomycin (BEP) [32]. More recently, these combinations have been supplanted by the less toxic carboplatin plus paclitaxel doublet once paclitaxel was identified to have activity [33]. Despite the historical use of chemotherapy in the treatment of higher stage disease, recent evidence from the NCD has shown no increased in survival in Stage II–IV disease [27].

Endocrine therapies

Endocrine therapies (see the next section below) were initially introduced with the rationale of interrupting hormone receptor signaling to achieve antitumor effect in a mechanism that is analogous to what is done in the treatment of hormone receptor positive breast cancer. Initial regimens included tamoxifen, and later progestins, in order to modulate estrogen receptor function. Later, regimens added gonadotropin-releasing hormone agonists to suppress ovarian function. More recently, AIs have had an increasing role in GCT management. Beneficial effects were seen in many case reports and these endocrine therapies have been used as treatments introduced principally as second-

Table 1. Early hormonal therapies in granulosa cell tumor using nonaromatase inhibitors.

	Therapy	# of patients	CR	PR	SD	PD	NA	CB	R	RR%	Ref.
Single agent GnRH hormonal therapy	Leuprolide	11	0	3	6	2	–	9	3	27%	[10,34–39]
	Goserelin	5	0	2	1	2	–	3	2	40%	[40–42]
	Total GnRH agonist	16	0	5	7	4	–	12	5	31%	
	GnRH antagonist (Ganirelix)	1	0	0	0	1	–	0	0	0%	[43]
Single agent hormone receptor agents	Megestrol acetate	11	1	3	5	2	–	9	4	36%	[42,44–46]
	Medroxyprogesterone	4	1	3	0	0	–	4	4	100%	[42,45,47]
	Tamoxifen	12	0	1	4	5	2	5	1	8%	[35,38,42,45,48]
	DES	1	0	1	0	0	–	1	1	100%	[49]
	Total hormone receptor agents	28	2	8	9	7	2	19	10	36%	
Hormonal therapy in combination	Leuprolide + tamoxifen	2	1	0	0	1	–	1	1	50%	[46,50]
	Goserelin + tamoxifen	1	0	0	0	1	–	0	0	0%	[40]
	Megestrol + tamoxifen	2	1	0	0	1	–	1	1	50%	[38,51]
Pooled non-AI hormonal therapies		50	4	13	16	15	2	33	17	34%	

AI: Aromatase inhibitor; CB: Clinical benefit; CR: Complete response; DES: Diethylstilbestrol; NA: Not available; PD: Progressive disease; PR: Partial response; R: Response; RR: Response rate; SD: Stable disease.

or third-line therapies after failure of one or more lines of chemotherapy. Thus, to date, endocrine therapies have played no role in adjuvant therapy after initial surgery.

Endocrine therapies overview

Studies prior to AIs

Non-AIs were some of the initial hormonal therapies used to treat GCTs. Treatment regimens included GnRH therapy (leuprolide, goserelin and ganirelix), hormone receptor acting agents such as megestrol acetate, medroxyprogesterone, tamoxifen and diethylstilbestrol (DES), and the above drug classes in combination. A total of 50 patients with known responses – either complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) – were described in the literature. Most of these cases were studied before the widespread clinical development of AIs in cancer. Table 1 summarizes responses of to such non-AI drug regimens, documenting their biological activity based on response rates. Of 50 patients, 33 had clinical benefit (CB), which is defined by adding CR, PR and SD events. However, only 17 of 50 cases had a documented tumor response (R) which is defined by the frequency of CR and PR, as seen in Table 1, and displaying it as a percent response rate (RR).

Aromatase inhibitors

In contrast to historical endocrine therapies, AIs constitute the major focus of this review because of both compelling rationale supported by the pathognomonic FOXL2 mutation that activates aromatase, as well as their early success in small series and case reports. Initially AIs were investigated in recurrent GCTs prior to the discovery of the pathognomonic mutation linking upregulation of aromatase with GCT. Previous reviews of the literature have shown a 71% (95% CI: 52–85%) pooled overall response rate to hormone therapy [52]. Van Meurs *et al.* review and institutional experience with AIs in 2014 resulted in the most favorable experience of their patients achieving complete and partial responses [52]. The reason behind the effectiveness of AIs remains undefined: antiproliferative effects of AIs could occur via alterations of the hormone-saturated cellular environment or through direct dampening effect on the tumor aromatase activation [52]. A quote from the Van Meurs review (p203) summarizes these findings: “... AIs led to the most responses in patients with GCT, of which five CRs and four PR (overall response rate 100%). None of the nine patients treated with AI have progressed since the start of treatment (6–54 months) and six are without evidence of disease”. We began this manuscript because three out of three of our patients with recurrent disease after incomplete responses to chemotherapy have not progressed for 8–26 months while being treated with letrozole.

AIs used in GCT therapy include letrozole, anastrozole and exemestane. The result of single agent AI use in GCT is summarized in Table 2. In the literature, 25 cases of AI use in GCT have been described with known response types. These cases and the cases of non-AI hormonal therapy use in GCT treatment can be found in the Supplementary Data. Seven of 25 cases had a CR, five of 25 have had a PR, and seven more had SD. 19 of 25 have had CB (CB = CR+PR+SD). In addition, reports in the literature have described successful rescue of the patients with letrozole after use of anastrozole, suggesting that letrozole may be a more potent AI for GCT treatment [53].

Table 2. Steroidogenesis inhibitor therapy, including aromatase inhibitor and antiandrogen, use in granulosa cell tumor.

Aromatase and other steroidogenesis inhibitors	# of patients	CR	PR	SD	PD	CB	R	RR%	Ref.
Letrozole	14	4	2	5	3	11	6	43%	[38,42,48,53–57]
Anastrozole	9	3	3	1	2	7	6	67%	[36,42,53,56,58,59]
Exemestane	1	0	0	0	1	0	0	0%	[48]
Unknown AI	1	0	0	1	0	1	0	0%	[42]
Total AI	25	7	5	7	6	19	12	48%	
Other: Single agent steroidogenesis inhibitor/antiandrogen (Ketoconazole)	1	0	0	1	0	1	0	0%	[60]

AI: Aromatase inhibitor; CB: Clinical benefit; CR: Complete response; NA: Not available; PD: Progressive disease; PR: Partial response; R: Response; RR: Response rate; SD: Stable disease.

However, a difference in efficacy between these two AIs including CB between letrozole and anastrozole (CB 11 out of 14 for letrozole and CB 7 out of 9 for anastrozole) is not apparent from these reports. AIs have also been used combined with other hormonal therapy: exemestane use was reported in one case as monotherapy and in combination with everolimus, emulating a strategy used in breast cancer. In this last instance, the patient experienced radiographic progression of disease after 2 months [48]. Evidence favoring AIs over other hormonal therapies in GCT is derived not only from the seemingly higher RR% but the more compelling rationale with our deeper understanding of GCT biology. These recent reports support further long-term investigations to induce regression of metastatic disease. Our recent experience using these drugs following PR to chemotherapy raises the possibility that their continued use lead to prolonged SD, and – unlike chemotherapy – do not lead to an increasingly aggressive phenotype.

Recently, other steroidogenesis inhibitors have also been tried in the treatment of GCTs. The use of ketoconazole, a CYP17 inhibitor, was shown to achieve stabilization of disease in one case [60]. This case is also presented in [Table 2](#) because the proposed mechanism of action is similar to that of an AI. Ketoconazole may also modify the hormonal environment of the tumor by blocking the CYP17 enzyme and thus block the production of androstenedione. It is also known that *CYP17A1*, like *CYP19A1*, is a transcriptional target of *FOXL2* [61].

Conclusion

Based on the collated data on AIs, it seems that their likelihood to induce response in GCTs may at least be non-inferior to non-AI hormonal therapies. Given the RR% to AIs, their significantly lower side effect profile compared to that of chemotherapy, and the recent findings that chemotherapy does not result in increased survival in metastatic disease, AIs, in our view, may be a viable therapeutic alternative to chemotherapy. Moreover, one should emphasize the expected risks and benefits of therapy to patients when deciding on treatment options for metastatic adult-type GCTs.

Future perspective

Although the data on hormone therapy use in GCTs are mostly retrospective – thus hindered by ascertainment bias – and these experiences in the utilization of AIs have not been validated by prospective clinical trials, one may anticipate increasing possibilities for such endocrine therapy in treatment of this disease. Moreover, one can envision that metastatic tumors showing lower proliferative rates, perhaps identified by low fluoro-deoxyglucose uptake on positron/computed electron tomography scans, may in the future be initially treated by hormonal therapies rather than the usual chemotherapy. On recurrence, rather than continuing chemotherapy until resistance sets in – a frequent paradigm in recurrent GCT – switching to endocrine therapy after an initial response to chemotherapy is worth consideration. It is noteworthy that CB-following AIs should be viewed as having better prospects of altering long-term outcomes, while those continuing on chemotherapy with SD incur greater cumulative toxicities. Moreover, sequential lines of chemotherapies upon progression may contribute to the evolution from an initially slow-growing neoplasm to ultimately life-threatening aggressive growths.

Progress in the treatment of GCT has been sought by leveraging our understanding of tumor biology coupled with emerging clinical experience with various staging and therapeutic modalities. In ClinicalTrials.gov listing under GCT and sex-cord stromal tumors, one finds an active recruiting registry, a planned study of bevacizumab,

a biological study of the effect of circulating androgens on response, two studies terminated early of ketoconazole and of orteronel, and two chemotherapy studies – one of them currently accruing. Unfortunately, the rarity of these tumors captures little attention from pharmaceutical companies, compounding the difficulties in making much headway in further exploring both clinical and biological leads.

These challenges are reinforced by the recently published NCD study. This retrospective cohort analysis of 2680 women with GCT from 1998 to 2013 emphasized three emerging findings: initial surgical staging stands out as likely contributor of a better outcome; stage is a major prognostic factor for eventual survival; and no clear benefit is seen from adjuvant chemotherapy – albeit the diverse regimens and collection of such details are potential shortcomings [27,62].

Nevertheless, a shift in focus of our understanding of the pathogenesis of this malignancy has been occurring since the FOXL2 mutation and its link to activating steroidogenesis were described. We await further insights as to how suppression of aromatase impedes cancer progression, while activation of these pathways leads to a more aggressive malignancy. The absence of improvement in survival from the retrospective cohort study by using chemotherapy is an added incentive to prospectively explore hormonal interventions.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/ije-2017-0021

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