RARE NON-EPITHELIAL OVARIAN NEOPLASMS:

PATHOLOGY, GENETICS and TREATMENT

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KEY WORDS: Small cell carcinoma of the ovary, hypercalcemic type, ovarian sex cord-stromal tumours, SMARCA4, DICER1, FOXL2

ABSTRACT

Rare non-epithelial ovarian neoplasms have posed management challenges for many years. Their rarity means that most specialist practitioners will see one such case every several years, and most generalists may never see a case. The first step in management is to establish the correct diagnosis and this may necessitate specialist pathology review. Here, we review recent developments in the pathology, genetics and treatment of small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) and sex cord-stromal tumours. Pathologically, these tumours often display morphological overlap with other neoplasms; for example, SCCOHT overlaps with many other “small round blue cell” tumours. Specific immunohistochemical stains, while useful, may not always be definitive. The discovery of somatic mutations in FOXL2 (adult granulosa cell tumours) and germline and somatic mutations in DICER1 (Sertoli-Leydig cell tumours) and SMARCA4 (SCCOHT) have demonstrated the value of molecular investigation as an adjunct to traditional histopathological approaches. In addition, the presence of germline mutations in a significant proportion of some of these neoplasms points to the need for genetic counselling and testing, offering the prospect of prevention and early diagnosis. Treatment of these rare tumours, as a group, should be on the basis of sound oncological principles, given that level 1 evidence will almost always be lacking. The rationale for experimental therapies must be clearly established. In view of the complex issues involved in the management of these conditions, expert opinion in pathology, genetics and treatment may be essential to offer the patient and her family the best chance of a good outcome.

INTRODUCTION

The various neoplasms covered in this review all comprise non-epithelial tumours and mainly, but not exclusively, those in the sex cord-stromal category. Previously there was little known regarding the underlying molecular abnormalities in the neoplasms discussed but in recent years there have been significant advances regarding this. This review is aimed at practitioners in medical genetics, gynaecological oncology, pathology and allied specialties. The main purpose of this review is not to review all that is known about the various tumour entities we discuss, but rather draw attention to the most recent developments, and to show how an inter-disciplinary model of case management, involving representatives from genetics, pathology, radiation and medical oncology and gynecology-oncology can be invaluable in managing problematic cases.

Each tumour will be discussed in detail, but by way of introduction and summary, here we list 15 general points from the perspective of a pathologist, geneticist and oncologist.

1 These are all rare or uncommon neoplasms such that it is possible that an individual pathologist or an oncologist may not even see one in his/ her diagnostic practice in their working life.

2 Many of these neoplasms and those in the differential diagnosis occur predominantly in young women, can be aggressive and require specific chemotherapeutic agents making a correct diagnosis imperative.

3 Given the rarity of these neoplasms, specialist review and confirmation of the diagnosis by an expert gynaecological pathologist is advised.

4 These neoplasms tend to exhibit morphological overlap with other tumours; for example, the morphological features of small cell carcinoma of the ovary,

hypercalcemic type (SCCOHT) overlap with a wide variety of other “small round blue cell neoplasms” and Sertoli-Leydig cell tumours (SLCTs) may exhibit significant morphological overlap with other sex cord-stromal tumours (SCSTs) and also epithelial neoplasms.

5 Immunohistochemical markers while of use in diagnosing a SCST are of no value in distinguishing between the various neoplasms in this group.

6 Molecular investigation may in some cases be essential in establishing a diagnosis.

7 Specific immunohistochemical markers may aid greatly in diagnosis; for example, SMARCA4 (BRG1) immunohistochemistry in the diagnosis of SCCOHT.

8 Establishing a diagnosis of some of the neoplasms discussed should result in consideration of a familial tumour syndrome which may require germline mutation analysis and ultimately result in screening of family members.

9 Genetic counselling should be considered for some SCSTs (for example SLCTs) and all SCCOHT as up to 50% of these tumours may arise in mutation carriers.

10 Little is known about the penetrance (age-dependent risk) for most of the conditions covered here, so consultation with experts is recommended.

11 Sequencing of both blood and tumour DNA may be of value in helping to determine risks to both the patient and her relatives.

12 While all in oncology are committed to the delivery of treatments that are “evidence-based”, to have Level 1 evidence from randomised trials as the sole arbiter of the management of patients with rare tumours is not only an impossibility, it is at best an absurdity and at worst discriminatory.

13 For slowly evolving tumours, repeat early surgery for relapses is more important than systemic therapy and should be instituted at a time of balance between too early for

small disease and too late when extensive, and hence, high-risk, surgery becomes necessary.

14 If the tumours show aggressive growth, a suitable chemotherapeutic regimen should be administered, but for two cycles only before evaluation, preferably including positron emission tomography; all chemotherapy should be stopped immediately if refractory to two lines or remissions are very short-lived.

15 Targeted therapy e.g. hormone, anti-angiogenic, checkpoint immunotherapy and molecular targeting must have proven biological rationale e.g. ER positive, VEGFR known to be present, increased neo-antigen load, EGFR expression. The rules for assessment of response to targeted therapy should be the same as for traditional chemotherapy.

SMALL CELL CARCINOMA OF OVARY, HYPERCALCEMIC TYPE (SCCOHT)

Clinical features of SCCOHT

SCCOHT is a rare tumour, with less than 500 cases reported in the world literature. Until 2014, little was known of its etiology, and its rarity made it difficult to study from an epidemiological perspective. As a tumour, SCCOHT has several notable features. Firstly, it affects young females; the youngest reported patient is 14 months[1] and the mean age at diagnosis is approximately 25 years[2]. Secondly, it is associated with a very poor outcome; even in early-stage disease only about one-third of affected women survive 5 years. Our recent analysis of all available cases[3] suggests that if treatment can prevent relapse within 5 years of diagnosis, then cure is very likely. Thirdly, SCCOHT is associated with hypercalcemia in approximately two thirds of cases[2].

Pathology of SCCOHT

In a large majority of cases, the tumour is unilateral, although familial cases may be bilateral. Grossly these are usually large predominantly solid white or cream coloured neoplasms, often with cystic foci and areas of haemorrhage and necrosis.

The histological features are typically of a relatively monotonous population of tumour cells with various architectural patterns set in a generally inconspicuous stroma[4]. There is usually predominantly a diffuse sheeted architecture but the neoplastic cells also occasionally grow in nests, cords or trabeculae. Follicle-like structures containing eosinophilic, or more uncommonly basophilic, fluid are present in most cases (figure 1a); these are a characteristic histological feature but are not pathognomonic since they are seen in some other neoplasms which are in the differential of SCCOHT[2, 4]. The tumour

cells usually have minimal cytoplasm resulting in a “small round blue cell” appearance. In approximately 40% of cases, a variably prominent component of large cells with abundant eosinophilic cytoplasm is present. The large cells typically contain abundant glassy eosinophilic cytoplasm with eccentric large pale nuclei with prominent nucleoli, resulting in a rhabdoid appearance[4]. When the large cells predominate or are exclusive, the tumour is rather confusingly referred to as the large cell variant of SCCOHT.

Genetic Susceptibility and Molecular Events in SCCOHT

Until recently, little was known regarding the histogenesis of SCCOHT and in the 2014 World Health Organization (WHO) Classification, it is included in the category of miscellaneous neoplasms[5]. In the last few years, four groups published papers showing that both germline and somatic mutations in the gene *SMARCA4*, encoding an ATPase containing member of the chromatin-remodelling family of proteins, are present in nearly all cases of SCCOHT (reviewed in ref[6]). This was a critical breakthrough, because as discussed elsewhere, SCCOHT can be very difficult to diagnose. The biallelic deleterious (usually predicted to be protein-truncating) mutations in most cases of SCCOHT result in loss of expression of SMARCA4 within the tumour cells. The significance of this is discussed further below.

What is very unusual about the genomics of SCCOHT is that SMARCA4 is the only recurrently mutated gene in this tumour, and moreover very few other genes are mutated at all in these neoplasms[6]. A recent study has illustrated this by comparing the mutation profile of high grade serous carcinoma of the fallopian tube/ovary with SCCOHT; these two tumours have very different mutational and epigenomic profiles[7]. Interestingly, the tumour that

SCCOHT seems to most resemble from a pathological and molecular viewpoint is the pediatric brain tumour, atypical teratoid/rhabdoid tumour (AT/RT)[7]. SCCOHT is in no way genetically related to other tumours called small cell carcinoma, such as small cell lung carcinoma and it is clear from the recent molecular studies that it is not an epithelial neoplasm[6].

It is uncertain what proportion of SCCOHT are attributable to germline *SMARCA4* mutations, but the number may be as high as two-fifths (Witkowski et al, manuscript submitted). This means that all females with SCCOHT should be referred to a genetics service. The risk for SCCOHT in carriers of germline SMARCA4 mutations is not known, but it is likely to be at least 20% and possibly much higher. Ascertainment bias and the rarity of the condition make it difficult to provide precise estimates. The question of whether to offer preventive oophorectomy to young carriers of *SMARCA4* mutations is a challenging area[8], especially since there are no data to suggest that ovarian surveillance is effective.

Immunohistochemistry of SCCOHT

Up until recently there were no specific immunohistochemical markers of SCCOHT[9-12]. Given the discovery that *SMARCA4* is mutated in almost all cases of SCCOHT[6], this has resulted in the development of antibodies which are useful in diagnosis and in the distinction from the many mimics. There is loss of SMARCA4 (BRG1) immunoreactivity secondary to mutation in almost all cases of SCCOHT (figure 1b), although a very small minority exhibit retention of nuclear immunoreactivity; occasional tumours exhibit loss of SMARCB1 (INI1) rather than SMARCA4[13, 14]. Positive staining of endothelial, stromal and inflammatory cells acts as a positive internal control and this should be present before

accepting loss of staining in the tumour cells as indicative of SCCOHT. Although there can be heterogeneity of staining in some other neoplasms in the differential diagnosis, there is usually at least focal retention of nuclear immunoreactivity. Some ovarian clear cell carcinomas exhibit loss of SMARCA4 staining but these do not enter into the differential diagnosis of SCCOHT[13, 14]. A single ovarian malignant melanoma has been reported to exhibit loss of SMARCA4 staining[14]. Since this is a neoplasm which may enter into the differential diagnosis of SCCOHT, this represents a potential diagnostic pitfall. However, the recent finding that dual loss of both SMARCA4 (BRG1) and SMARCA2 (BRM) protein expression is highly specific for SCCOHT[15] should mean that such pitfalls can now be avoided.

Differential Diagnosis of SCCOHT

SCCOHT is the prototypical ovarian neoplasm composed predominantly or exclusively of small round cells with scant cytoplasm (so-called “small round blue cell tumour”). The differential diagnosis of ovarian small round blue cell tumours is wide (table 1) and pathologists commonly struggle with these neoplasms due to their rarity and overlapping morphology and immunohistochemistry[16, 17]. Many of these neoplasms occur predominantly in young women, are highly aggressive and require specific chemotherapeutic agents making a correct diagnosis imperative. In diagnosing the various tumour types, immunohistochemistry and molecular studies may be of value. While typical SCCOHT is part of the differential diagnosis of a small round blue cell tumour, the large cell variant may be confused with a variety of neoplasms composed of large cells such as undifferentiated carcinoma and malignant melanoma. It should be noted that the WHO 2014 Classification includes a category of ovarian small cell carcinoma of pulmonary type[5,

18]; this is a small cell carcinoma of neuroendocrine type (similar to those occurring in the lung and many other organs) and is in no way related to SCCOHT, although the similar terminology often results in confusion.

Treatment of SCCOHT

A general approach to the management of rare tumours is shown in box 1. Small cell carcinoma of the lung and indeed small cell tumours at other sites are generally sensitive to chemotherapy, particularly platinum-based regimens. SCCOHT is no exception and in many ways behaves very similarly to small cell carcinoma of the lung in that response rates are high but relapse is frequently seen. Stage 1 tumours are potentially curable whereas few patients with advanced stage disease survive. There are three controversies in the management of patients with early stage SCCOHT: firstly, the extent of surgery, secondly which chemotherapy regimen to use and finally whether or not adjuvant radiotherapy has a part to play.

The extent of surgery at presentation is a considerable issue as many of these patients are young and wish to preserve fertility. Usually in a young female with tumour seemingly confined to one ovary, fertility-sparing surgery, for example unilateral salpingo-oophorectomy and omentectomy, is performed following complete surgical staging. Most oncologists would administer adjuvant platinum-based chemotherapy following surgery. A major question, particularly in patients who wish to preserve their fertility, is whether or not radiotherapy is given adjuvantly and whether that radiotherapy is to the pelvis alone, pelvis and para-aortic area or the whole abdominal-pelvic region. Clearly pelvic radiotherapy is associated with gonadal failure and therefore to perform fertility-sparing surgery when

pelvic radiotherapy in the adjuvant setting is being contemplated is not in our view, a logical strategy.

A review of the experience at the MD Anderson has recently been published and this sets out their experience which very much mirrors that of these authors[19]. The commonest adjuvant chemotherapy used is a combination of platinum and etoposide. Our practice is to use BEP (bleomycin, etoposide, cisplatin) but other platinum-based regimens which are a variation on this have been used and occasionally carboplatin and paclitaxel is used. Some patients in the MD Anderson series received adjuvant radiotherapy as well as chemotherapy. Interestingly, in this series only 1 of the 12 long term survivors (The disease stages of these 12 patients were: 8 stage I, 2 stage II and 2 stage III) received no adjuvant treatment at all. The other 11 long term survivors all received adjuvant chemotherapy with a minority also being treated with radiotherapy after chemotherapy. These data suggest that radiotherapy may not be an essential addition to adjuvant chemotherapy in patients with early stage disease (see below).

However, there are also data to suggest that radiotherapy does have a part to play. Harrison and colleagues[20] analysed the results in 10 patients with stage 1 disease who had received BEP chemotherapy Five of six patients who received adjuvant radiotherapy after chemotherapy were disease-free whereas only one of four patients who did not receive adjuvant radiotherapy following BEP survived. The long term morbidity of radiotherapy on bowel and sexual function needs to be addressed in these patients during the consent process prior to the instigation of this treatment.

The arguments relating to the use of radiotherapy along with chemotherapy and perhaps surgery are completely different in patients whose disease has relapsed. It is the view of these authors that maximal therapy, including all 3 modalities, is justified because the risks of not being cured are extremely high when SCCOHT relapses.

OVARIAN SEX CORD-STROMAL TUMOURS (SCSTs)

Ovarian SCTS comprise a heterogeneous group of neoplasms, both benign and malignant (table 2). There may be considerable morphological overlap between the various SCSTs and also between SCSTs and a variety of non-sex cord-stromal tumours such that pathologists often struggle with the diagnosis and immunohistochemistry is often useful in diagnosis. With selected tumour types, we discuss the underlying genetic susceptibility and associated molecular abnormalities but we start this section with a general point regarding the role of immunohistochemistry in ovarian SCSTs.

IMMUNOHISTOCHEMISTRY OF OVARIAN SEX CORD-STROMAL TUMOURS

While immunohistochemistry is useful in diagnosing a SCST, it is of limited or no value in distinguishing between the various neoplasms in this category. Probably the best known markers of ovarian SCST are inhibin and calretinin, the former being more specific[21-27]. Although inhibin is positive in a high percentage of SCSTs, some cases are negative. This may shake the confidence of the pathologist and it is stressed that a diagnosis of a SCST can be confidently made in the absence of inhibin immunoreactivity. Other markers which may be positive in this group of neoplasms are steroidogenic factor 1 (SF1), FOXL2 (discussed later), CD56, CD99, melan A, CD10, Mullerian inhibiting substance and WT1[21-27]. However, all of these have limitations with regard to specificity and/or sensitivity and are of relatively

limited value in diagnosis. In the distinction between a SCST and an epithelial neoplasm, epithelial membrane antigen (EMA) is very useful since this is positive in most epithelial neoplasms but almost invariably negative in SCSTs.

OVARIAN GRANULOSA CELL TUMOUR

There are two distinct types of ovarian granulosa cell tumour, namely adult granulosa cell tumour (AGCT) and juvenile granulosa cell tumour (JGCT). Although AGCTs occur most commonly in the perimenopausal and early postmenopausal years and JGCTs in the first three decades, there is overlap in that AGCTs may occur in younger patients and JGCTs in older patients. It is stressed that the distinction between AGCT and JGCT is made on the basis of the morphological features and not the patient age.

ADULT GRANULOSA CELL TUMOUR

Clinical Features of AGCT

AGCT is the most common malignant ovarian SCST, although it accounts for only 1-2% of all ovarian neoplasms and approximately 5% of ovarian malignancies[28]. Presentation is usually with symptoms related to an ovarian mass but some patients present with hormonal manifestations, most commonly oestrogenic (such as abnormal uterine bleeding) but occasionally androgenic or progestogenic. Some patients present with pain due to tumour torsion or rupture. The vast majority of AGCTs are stage I at presentation and have a good long term survival, although recurrence is common. The overall 10 year survival for stage I neoplasms is in the region of 85 to 95%[29]. Recurrences may be multiple (in number and time) and often are detected many years following removal of the primary ovarian neoplasm; it is not uncommon for tumours to recur in excess of 10 or even 20 years

following initial surgical removal. The most important prognostic factor is tumour stage. However, almost all AGCTs are stage I at diagnosis (in older case series with a high incidence of advanced AGCTs, it is likely that many were misdiagnosed) and currently there are no features which predict with any reliability which stage I AGCTs are likely to recur and would be candidates for adjuvant therapy following surgical removal. The one possible exception is tumour rupture which has been shown to be a factor predicting tumour recurrence in the few studies where this has been examined[29]. Most patients with tumour recurrence will eventually die of disease. A recently published large single-centre retrospective analysis has shown that in AGCTs, surgical stage was the only independent factor for the progression-free survival[30].

Pathology of AGCT

Histologically AGCT is characterized by the presence of regular cells with ovoid or round nuclei containing pale chromatin and sometimes nuclear grooves (figure 1c); the cytoplasm is usually scant with the exception of luteinized variants. Although nuclear grooves have been considered a characteristic feature of AGCT, they are not seen in every case and are often not prominent and the pathologist should not rely on the presence of these to make a diagnosis of AGCT; moreover, nuclear grooves are seen in many other neoplasms. The tumour cells in AGCT may be arranged in a variety of architectural patterns, which may be pure or mixed within a particular neoplasm; these include microfollicular, macrofollicular, trabecular, insular, diffuse (sarcomatoid), watered silk (parallel rows) and gyriform (zigzag cords). The best known architectural pattern is the microfollicular Call-Exner body; however, these are seen in a minority of AGCTs and, like nuclear grooves, their importance in diagnosis has been overemphasised.

Genetic Susceptibility and Molecular Events in AGCT

There is no known genetic susceptibility to AGCT, and there are no reports in the literature of families with multiple AGCTs. Until recently, there was little known regarding the underlying somatic molecular events in AGCT but several recent studies have identified a somatic missense mutation in codon C134W (402C→ G) of the FOXL2 (*forkhead box* L2) gene in approximately 95% of AGCTs[31-33]. The FOXL2 gene encodes a transcription factor required for ovarian development, in particular granulosa cell function and ovarian follicle development. *FOXL2* mutation is rare in SCSTs other than AGCT[31-33] and it has been suggested that these may represent misclassified AGCTs. Correct diagnosis of AGCT is important for prognostication given the potential for late recurrence and metastasis which is not a feature of other SCSTs. Given that FOXL2 mutation is an extremely sensitive and quite specific molecular marker of AGCT, testing may be extremely useful in helping to confirm or refute a diagnosis of AGCT in problematic cases.

While FOXL2 mutations are largely, but probably not exclusively, restricted to AGCT, many SCSTs of other types exhibit positive nuclear staining with antibodies against FOXL2. Therefore, while FOXL2 immunohistochemistry is useful in confirming a SCST since almost all non-sex cord-stromal tumours are negative, it is of limited value in distinguishing between the various tumour types[34].

JUVENILE GRANULOSA CELL TUMOUR

JGCTs may present with symptoms related to an ovarian mass or with hormonal manifestations such as isosexual precocious puberty[35]. There are differences in clinical

behaviour between JGCT and AGCT in that the former is less likely to recur or metastasise but when this does happen it usually occurs early within a few years[35]; this is in contrast to the propensity of AGCT for late recurrence and metastasis. Hypercalcemia has been reported in occasional cases[36-38].

Almost all JGCTs are unilateral. Most grow as diffuse sheets of tumour cells with hyperchromatic nuclei and relatively abundant eosinophilic or luteinised cytoplasm. Follicles of variable size and irregular shape are common containing basophilic or eosinophilic fluid (figure 1d). There may be abundant mitotic activity and moderate or even severe nuclear atypia.

SCCOHT is an important differential diagnostic consideration. Both neoplasms typically affect the same age group but, in striking contrast to JGCT, SCCOHT follows a highly aggressive course. Hypercalcaemia is highly suggestive of SCCOHT but is only present in about two-thirds of cases, and has rarely been reported in JCGT (see above). Conversely, oestrogenic manifestations point to JGCT but they are not present in all cases.

Immunohistochemistry is of considerable value in problematic cases since SCCOHT exhibits loss of nuclear immunoreactivity with SMARCA4 and is often positive with EMA while JGCT is positive with sex cord markers such as inhibin, negative with EMA and there is retention of nuclear staining with SMARCA4.

Genetic Susceptibility and Molecular Events in JGCT

JGCTs occasionally occur in a number of genetic syndromes, such as Olliers disease (multiple enchondromas)[35, 39-41] and the genetically-identical Maffucci's syndrome (multiple enchondromas associated with soft tissue hemangiomas)[42]. Amary et al[43] reported somatic mosaic *IDH1* and *IDH2* mutations, nearly always affecting Arg132 of *IDH1* or Arg172 of *IDH2*, in both Olliers disease and Maffucci syndrome, strongly suggesting that the JGCTs occurring in these patients are similarly caused by somatic mosaic mutations in these two genes. Somatic *DICER1* mutations are occasionally found in JGCT; in one study, one of 14 JGCTs were reported to have somatic "hotspot" *DICER1* mutations (see below)[44], but none were found in 4 JGCTs in another study[45]. Two JGCTs have occurred in patients likely to carry germline *DICER1* mutations[46], but to date no such germline-mutated cases have been published.

It is important to note that JGCT is different from AGCT in that *FOXL2* mutations are found only rarely in the former. In one study, "hotspot" activating GNAS mutations (at position 201) were reported in nine of 30 patients with JGCT[47]. Recently, the same group found in-frame tandem activating duplications of exon 3 of *AKT1* in 9/16 JGCTs diagnosed under the age of 15 years[48, 49]. RNA-Seq analysis showed patterns of gene expression supportive of an important role for this mutation in the pathogenesis of JGCT. Interestingly, this group also reported the presence of this activating duplication in a *FOXL2*-negative AGCT[48]. These findings show that AGCT and JGCT are in general molecularly distinct entities.

TREATMENT OF AGCT AND JGCT

The mainstay of management of patients with AGCTs is surgery. Fertility-sparing surgery, following complete surgical staging, is acceptable because the incidence of bilaterality is below 5%.

The sensitivity of AGCTs to platinum-based chemotherapy varies from 40-90%. Bleomycin, Etoposide, cisPlatinum (BEP) chemotherapy is the most commonly used regimen with response rates as high as 80%, although earlier studies of nonplatinum-based chemotherapy have shown response rates of 60-70% (reviewed by Kottarathil et al[50]). Taxanes have been introduced to regimens for AGCTs with response rates similar to BEP in newly diagnosed patients but there are no randomised data comparing BEP with platinum-paclitaxel combinations. Nonrandomised comparisons in patients with recurrent disease have suggested that there may be an advantage to BEP, but these results must be interpreted with great caution. The Gynecologic Oncology Group is currently conducting a randomized phase II trial of BEP versus carboplatin-paclitaxel in women with ovarian SCSTs who have not previously received chemotherapy in both the first line and relapsed setting (GOG 264, NCT01042522).

Some argue that there is a role for adjuvant chemotherapy in patients with advanced disease who have their tumour debulked either optimally or sub-optimally. While some authors have reported no recurrences in early stage disease following adjuvant BEP, it is clear that patients with early disease can be cured by surgery alone or at least have very long disease-free intervals. We do not suggest adjuvant chemotherapy for patients with early stage disease. Even in patients who have completely resected advanced disease, there

is a question as to whether or not postoperative chemotherapy should be given because many patients have slowly evolving tumours and there is merit in adopting a watch and wait policy to see whether the patient's tumour behaves in an indolent or aggressive fashion. For patients with indolent disease, repeated surgery with the institution of chemotherapy only with bulky disease that cannot be treated surgically, allows the patient to survive longer with good quality of life without the side effects of chemotherapy.

Angiogenesis inhibitors have also been used in recurrent disease because of the vascular nature of these tumours and both response and stabilisation of disease is reported. Another targeted approach under investigation involves monoclonal antibodies against tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors. Hormone therapies are not generally used in the adjuvant setting but do have a role for patients with relapse. Responses can be obtained and some are durable. The advantage of hormone therapy is the comparative lack of side effects compared to chemotherapy. There is no clear single mechanism of action that accounts for the beneficial effect of hormone therapy and most hormonal agents have shown some effect including medroxyprogesterone acetate, megestrol, anastrozole, letrozole and gonadotropin-releasing hormone agonists.

The precise role of radiotherapy is not defined. There are data which suggest an advantage with adjuvant radiation but the advantage appears to be relatively small and there is always the issue of the long term side effects of pelvic recurrence. Radiation is best kept in reserve for incompletely resected or single site non resectable recurrent disease where it can have a very useful palliative role.

The young age of onset and rarity of JGCTs of the ovary make decisions concerning fertility-sparing surgery difficult because some authors have claimed that radical surgery and even adjuvant radiotherapy and chemotherapy are associated with better outcomes, in particular high cure rates. However, not all retrospective series have shown this and considerable caution is required before young women are subjected to radical treatments that will affect their long term morbidity and quality of life. For stage 1A JGCT, there is no evidence that any treatment other than fertility-sparing surgery is of benefit and issues that relate to the administration of adjuvant chemotherapy or radiotherapy should only be considered in patients with Stage 1C or above disease; for these patients the evidence for adjuvant treatment is weak. We would not consider pelvic radiation because of its morbidity. However, for both AGCT and JGCT, in patients with Stage 1C disease some recommend adjuvant BEP in the presence of other poor prognostic features such as nuclear atypia, high mitotic index, aneuploidy, age > 40 years, but the significance of these factors is debated; we would not routinely use them or give adjuvant therapy to patients with a Stage 1C granulosa cell tumour[51-53].

SERTOLI-LEYDIG CELL TUMOURS

Ovarian Sertoli-Leydig cell tumours (SLCTs) occur at all ages but have a propensity to arise in relatively young patients with a mean age of approximately 25 years. Retiform variants usually occur in particularly young patients with a mean age of 15 years. Presentation may be with symptoms related to an ovarian mass or with virilisation. Most tumours are unilateral and confined to the ovary at diagnosis. The WHO Classification divides SLCTs into well, moderate (previously referred to as intermediate) and poorly differentiated and

retiform variants[5]. Moderate and poorly differentiated and retiform variants may contain heterologous elements.

Well differentiated SLCTs are composed of solid or hollow Sertoli cell tubules and variable numbers of Leydig cells (figure 1e). Moderately differentiated tumours often have a low power lobulated architecture and are characterized by cellular areas composed of cords, nests and tubules of immature Sertoli cells. Leydig cells are present which are often most apparent at the periphery of the cellular aggregates of Sertoli cells. Poorly differentiated neoplasms contain a significant amount of immature cellular mesenchymal tissue with high mitotic activity, often resembling an undifferentiated sarcoma. Establishing a diagnosis relies on the presence of more distinctive patterns of Sertoli cell elements and Leydig cells which may be revealed by extensive sampling. Retiform SLCTs have a characteristic gross appearance with large oedematous polyps, often resembling a hydatidiform mole or serous borderline tumour. The morphological features are of a network of elongated tubules and cysts, resembling the rete testis.

Heterologous elements occur in approximately 20% of SLCTs. The most common heterologous element is intestinal type mucinous epithelium Less common heterologous elements include rhabdomyoblasts, cartilage, hepatoid elements and foci of carcinoid tumour

Genetic Susceptibility and Molecular Events in SLCTs

The most important genetic susceptibility factor for SLCTs is germline *DICER1* mutations. In 2011, Rio Frio *et al* identified different pathogenic germline *DICER1* mutations in two

families containing women with both SLCT and multinodular goitre[54], confirming the genetic basis of an association that had been noted many years previously. Subsequently, somatic *DICER1* mutations were identified in the SLCTs arising in these germline-mutated cases[44], as well as somatic mutations in cases where germline *DICER1* status was not known. From this and subsequent studies, it appears that up to 60% of SLCTs carry somatic mutations in *DICER1*[44, 55-57]. The prevalence of germline *DICER1* mutations in SLCT is not known, but it may be as high as the somatic mutation frequency, and all patients with SLCT should be referred to genetics services. One of the most fascinating aspects of the mutations seen in SLCTs and other *DICER1*-related tumours is that they are almost entirely restricted to exons encoding the RNase IIIb domains of the protein. Moreover, nearly all the mutations are predicted to not truncate the protein; instead, they are missense mutations affecting the metal-ion binding residues within the RNase IIIb domain[44]. These mutations appear to have oncogenic roles[58], and function by altering the balance between 5p and 3p microRNAs[59, 60], which are the products of DICER1 cleavage of precursor microRNAs[61]. How these mutations lead to the highly specific, and generally very rare, manifestations of the DICER1 syndrome is not known.

While most ovarian SCSTs associated with *DICER1* mutations are SLCTs, there are also occasional reports of JGCT, Sertoli cell tumour, gynandroblastoma and unclassified SCST harbouring these mutations[56]. While the demonstration of *DICER1* mutations in these various neoplasms may well indicate that mutations occur in SCSTs other than SLCTs, it should be pointed out that there is significant morphological overlap between tumours in the SCST category and it is possible that some of these other neoplasms containing *DICER1* mutations represent misclassified SLCTs. A recent study of a small number of cases

suggested that gynandroblastomas (a SCST exhibiting both male and female differentiation) may encompass two distinct entities at the genetic level, those with a granulosa-like genotype exhibiting *FOXL2* hotspot mutations and those with a SLCT-like *DICER1* mutant genotype[56].

Treatment of SLCT

Patients with stage 1A well differentiated Sertoli-Leydig tumours do not require adjuvant therapy and can be managed by fertility-sparing surgery only following complete surgical staging. Moderately and poorly differentiated SLCTs and higher stage tumours should probably receive adjuvant chemotherapy if for no other reason that when relapse occurs, survival is low because relapsed Sertoli-Leydig tumours are incurable and often behave aggressively[62-64].

BEP is one of the most commonly used regimens for these tumours, although other platinum-based regimens have also been used such as cisplatin-doxorubicin-cyclophosphamide, carboplatin-epirubicin-etoposide, cisplatin-vinblastine-bleomycin and platinum-taxane.

OVARIAN SEX CORD TUMOUR WITH ANNULAR TUBULES (SCTAT)

This is an uncommon ovarian neoplasm which occurs either sporadically (most commonly) or in association with Peutz-Jeghers syndrome (PJS)[65, 66]. Those neoplasms associated with PJS occur on average at a slightly younger age than sporadic neoplasms. Sporadic neoplasms are almost always unilateral and present with menstrual irregularities or symptoms related to an ovarian mass. In contrast, those neoplasms associated with PJS are

almost always bilateral and multifocal within the same ovary. Often they are incidental microscopic findings in patients known to have PJS; it has been suggested that microscopic SCTATs are almost invariably seen in the ovaries of patients with PJS.

Both PJS-associated and sporadic SCTATs are composed of well circumscribed rounded nests of cells with punched-out spaces containing hyalinized basement membrane-like material which may be focally calcified (figure 1f). The nuclei are characteristically located at the periphery of the nests. Uncommonly, small foci resembling AGCT or Sertoli cell tumour are present, especially in non-PJS associated cases.

The prognosis of SCTATs in patients with PJS is excellent with a usual benign clinical course, although occasional neoplasms have behaved in an aggressive manner[66]. Sporadic SCTATs have a potential for malignant behaviour and extra-ovarian spread (one fifth of cases) which cannot be reliably determined by microscopic examination[66].

When diagnosing a SCTAT, the pathologist should raise the possibility of PJS syndrome; probably at least a third of all SCTATs occur in the context of PJS[66]. Establishing a diagnosis allows screening to detect other neoplasms which potentially occur in patients with PJS, including cervical gastric-type adenocarcinomas.

Germline mutations in *SKT11* are the underlying cause of PJS[67]. Loss of heterozygosity at 19p13.3, including *SKT11*, is seen in SCTATs associated with PJS[68]. Point mutations in the *SKT11* gene have not been detected in PJS-related or PJS unrelated SCTATs[68] or in SCSTs in general[69]. The lifetime risk for SCTAT in female *STK11* mutation carriers is uncertain. In a

large retrospective cohort study that included 226 female *STK11* carriers, only two ovarian tumours were recorded[70], suggesting a low risk, whereas a previous meta-analysis found a risk for ovarian tumours of 21%[71], mandating yearly pelvic ultrasonography[72]. This could be regarded as unjustified given the excellent prognosis of SCTAT in PJS, and has recently been rejected as a recommended surveillance strategy[73].

CONCLUSIONS

In this review, we have discussed the key pathological, genetic and management aspects of selected uncommon ovarian non-epithelial tumours. As a group, they often present challenges to both the pathologist, from a diagnostic viewpoint, and to the oncologist from the treatment perspective. Recent molecular evaluation of patients with these neoplasms (and the tumours) has resulted in the discovery of somatic and genetic mutations resulting in important insights which may aid both the pathologist and the oncologist. It is likely that further molecular characterization of these tumours, combined with innovative randomized "basket" trials, will lead to the development of specific targeted therapies and significant improvement in the outcome for the more aggressive tumours in this group, such as SCCOHT.

Acknowledgments

We thank Talia Boshari for her help in producing this manuscript. Work in WDFs lab of relevance to this work was supported by a Canada Cancer Research Society Institute Innovation Award and Alex's Lemonade Stand Foundation. MG acknowledges NHS funding to the NIHR Biomedical Research Centre at the Royal Marsden Hospital and Institute of Cancer Research.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Figures and Tables

Figure 1. SCCOHT composed of sheets of small round blue cells puntuated by many follicle-like spaces (a). Loss of nuclear immunoreactivity with SMARCA4 (retention of staining in endothelial cells serves as an internal positive control) is useful in diagnosis of SCCOHT (b). AGCT composed of regular cells with vesicular nuclei, some containing grooves; microfollicular Call-Exner bodies are present (c). JGCT composed of cells with abundant cytoplasm arranged in diffuse sheets with intermediate sized follicles (d). Well differentiated SLCT composed of Sertoli cell tubules with surrounding Leydig cells with eosinophilic cytoplasm (e). SCTAT composed of well demarcated nests of cells with punched out spaces containing hyalinized basement membrane-like material (f).

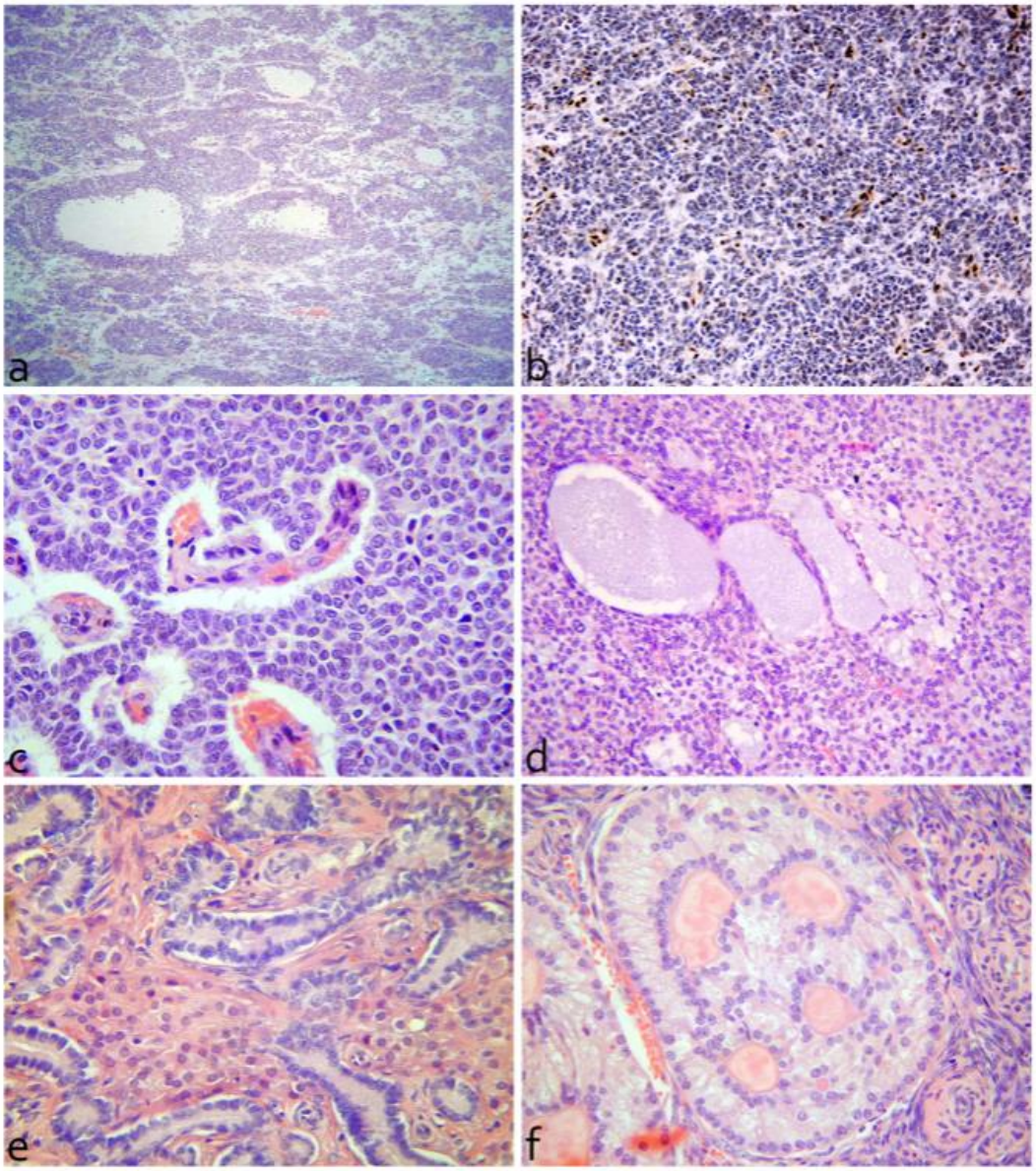

Table 1: Differential diagnosis of ovarian neoplasms composed of small round blue cells. This is not an exhaustive list since occasionally other neoplasms may enter into this differential diagnosis.

Primary
Granulosa cell tumors
Sertoli-Leydig cell tumor (moderately and poorly differentiated)
Luteinized thecoma associated with sclerosing peritonitis
Endometrial stromal sarcoma
Carcinoid tumor
Small cell carcinoma, hypercalcaemic type
Small cell carcinoma, pulmonary type
Malignant melanoma
Undifferentiated carcinoma
Immature teratoma
Lymphoma/ leukaemia
Primitive neuroectodermal tumour/ Ewing family of tumors
Rhabdomyosarcoma
Undifferentiated sarcoma
Metastatic
Endometrial stromal sarcoma
Breast carcinoma
Intra-abdominal desmoplastic small round cell tumor
Malignant melanoma
Carcinoid tumor
Small cell carcinoma
Primitive neuroectodermal tumour/ Ewing family of tumors and neuroblastoma
Rhabdomyosarcoma

Table 2: WHO 2014 Classification of ovarian sex cord-stromal tumours. ([a] Moderately and poorly differentiated and retiform variants may contain heterologous elements. [b] NOS = not otherwise specified).

Pure stromal tumours	**Pure sex cord tumours**	**Mixed sex cord-stromal tumours**
Fibroma	Adult granulosa cell tumour	Sertoli-Leydig cell tumours (well differentiated, moderately differentiated, poorly differentiated, retiform)[a]
Cellular fibroma	Juvenile granulosa cell tumour	Sex cord-stromal tumours, NOS[b]
Thecoma	Sertoli cell tumour	
Luteinized thecoma associated with sclerosing peritonitis	Sex cord tumour with annular tubules	
Fibrosarcoma		
Sclerosing stromal tumour		
Signet-ring stromal tumour		
Microcystic stromal tumour		
Leydig cell tumour		
Steroid cell tumour		
Steroid cell tumour, malignant		